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**Sexual Motivation and Mate Choice in the Female Smooth
Newt, *Triturus vulgaris***

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Thesis submitted for the degree of Doctor of Philosophy at the Open University,
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ABSTRACT

This thesis describes a study of sexual motivation and mate choice in the female smooth newt, *Triturus vulgaris*. Chapter one introduces the species' natural and life histories and sexual behaviour. It also describes the different approaches (causal and functional) that have been employed and explains why a female perspective has been particularly emphasised.

Chapter three reports the results of the preliminary study of female sexual motivation which provided the basis for further investigation. Chapter four discusses some of the results of the follow-up study. Female sexual responsiveness was monitored over a complete breeding season. The relationship between inseminations and egg-laying was investigated. This chapter essentially looked at female sexual responsiveness from a functional perspective. It was found to vary greatly, and was affected by egg-laying and time of season.

Chapter five discusses female sexual responsiveness in the same study reported in Chapter four, but from a causal perspective. Female sexual behaviour was described in detail, behaviour patterns were defined and motivation scores were devised to examine variation over the season. Chapter six examines female mate choice. Aspects of male morphology, spermatophore pick-up and partner familiarity were investigated. Females were found to show discrimination for larger-crested males, but this discrimination varied and was related to sexual motivation. Chapter seven reports an investigation into how females perceive the stimuli presented to them by the male during his display. The relative importance of visual and olfactory stimuli was studied, as well as the effect of the combination of both sets of cues.

Chapter eight re-examines the value to this area of research of using the different approaches and perspectives employed here and discusses the results of all the experiments performed.

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CHAPTER 1

INTRODUCTION

1.1 The Natural History of the Smooth Newt

The objective of the study described here was to investigate sexual motivation and mate choice in the female smooth newt, *Triturus vulgaris*. To provide a starting point and background for the study that follows, the natural history and life history of the smooth newt, will be briefly reviewed in the following section.

1.1.1 Immigration to the Pond

Adult smooth newts spend a large part of the year out of water but very little is known about this terrestrial phase of their existence. The breeding season begins towards the end of February or in early March when daylength increases and the temperature begins to rise. A study by Harrison et al (1983) showed that migration was related to both temperature and rainfall but Verrell & Halliday (1985) found that although temperature was important, rainfall did not appear to be so. The newts' aquatic phase, which constitutes their breeding season, has been the subject of intensive behavioural and ecological study (e.g. Baker 1990a, 1990b; Dolmen 1983; Griffiths 1984, 1985; Halliday 1974, 1975, 1976; Verrell & Halliday 1985; Verrell et al 1986).

Various studies have tried to determine whether males arrive in the pond at a different time, or at the same time as the females. This is of interest since it may be adaptive for males to arrive earlier, if one assumes that their mating strategy is to inseminate as many females as possible. According to Trivers' (1972) parental investment hypothesis, males that do not have a role in parental care 'invest' less in each of their numerous small gametes than the female does in each of hers, which are larger, relatively few in number and have to be cared for in some way. In the case of female newts, this involves the time-consuming task of selecting egg-laying sites and

laying between 200 and 600 eggs. The variation in clutch size was found to be related to body size (Baker 1990a). Trivers (1972) suggested that the males' strategy should be to try and inseminate as many females as possible and that females should be choosy about with which male they mate. Thus there is some basis for suggesting that males should arrive earlier than females, so they are ready to intercept the females as soon as they begin to arrive in the water.

Different reports provide conflicting information about whether newts do, in fact, exhibit protandry. For example, Verrell & Halliday (1985) found that there was no difference in arrival time for the two sexes, but Griffiths (1984) found that males arrived earlier. Personal observation of the two ponds used in this study (see Chapter 2) indicated a similar situation to that described by Griffiths, whereby, over a number of evenings, males arrived in abundance on the earlier evenings and more females arrived on later evenings. However these observations only represent a very small part of the migration period and were not consistent from year to year. Verrell & Halliday (1985) reported that immigration occurred over a long period of time, whereas Harrison et al (1983) found more of a mass migration.

It is clear that there is no definite pattern of immigration that describes the situation at every pond. Perhaps this should be expected since each pond is unique in terms of its location, physical and chemical characteristics, flora and fauna, and surrounding habitat. Similarly, the weather conditions leading up to migration time are different from year to year. Long term studies of individual ponds are needed to reveal any patterns that underlie immigration. At present only temperature seems to be consistent in influencing the movements of newts to their breeding ponds.

1.1.2 The Aquatic Phase

On reaching the pond a newt becomes readapted for its aquatic existence. The shape of the tail becomes more flattened to aid swimming; the jaws adapt to enable food to be taken in by a different method; the skin becomes modified to act as a respiratory surface; changes in the visual and olfactory systems occur so that the

animal can see and smell in a liquid medium and the lateral line system recrudescens (Halliday 1972).

In addition to these changes the male develops a number of secondary sexual characteristics. Both sexes are olive-greeny brown with a yellow or orange belly. The skin has dark spots in both sexes but the males develop much larger spots at this time. The male also develops a large dorsal crest which runs from behind the head, along the back and into the tail, becoming part of the tail blade. The lower edge of this tail blade has an orange-red stripe running along it and just above this, a band of silvery-blue coloration develops. This band of colour is conspicuous during the male's courtship display (Halliday 1974; Verrell 1981), especially during the movements *fan* and *whip* (terms defined in Halliday 1974) when the tail is moved rapidly (pers. obs.). The male also develops fringes of skin along his hindlimb digits (toe flaps) which may serve to stabilize his display movements (Halliday 1975; Halliday & Joly 1981; Beebee 1981).

According to Tinbergen & Ter Pelkwijk (1938) the female's upper jaw is better adapted for the aquatic feeding mode than that of the male. The flaps that develop on either side of the jaw, to enable water to be drawn into the mouth, are more pronounced in the female. Thus, as this occurs only in the female and only in the breeding season, this feature could be considered a female secondary sexual characteristic, but perhaps of more ecological than sexual significance.

Bell (1977) suggested that males are probably ready to breed as soon as they enter the water, but that females need to be in the water for about forty days to mature their full clutch of oocytes. However, more recent studies (Hagstrom 1979; Verrell & Halliday 1985) suggested that this is not necessary, but indicate that the length of time between entering the water and ovulation can vary greatly (between 14 and 7 days depending on how early the pond was reached). The present study also indicates that females can be ready to mate immediately after they enter the pond and that they begin egg-laying soon after.

Several workers have tried to investigate the sex ratio of newts present in a breeding population. Bell (1977) estimated a sex ratio in favour of females, Blab & Blab (1981) found a 1:1 ratio and Griffiths (1984) reported a preponderance of males. Verrell & Halliday (1985), in a study aimed at trying to assess the intensity of sexual selection in the wild, obtained an estimate of the Operational Sex Ratio (OSR) (Emlen & Oring 1977), which is more meaningful than the overall sex ratio when considering mating dynamics. The study found that the OSR in fact changed over the course of the breeding season and was related to female responsiveness and egg-laying activities. These factors were examined in detail in the present work and will be discussed in more detail later.

Sexual activity in the pond tends to occur mainly at the beginning of the season (Verrell & McCabe 1988; present study). Within a daily timescale, there is a peak of courtship activity around dusk (Griffiths 1985), but some courtship also occurs at dawn (Dolmen 1983).

Females begin ovipositing a few days after insemination and oviposition takes place mainly at night (Dolmen 1976). Dolmen (1976) and Pecio (1992) both reported that the beginning of egg-laying occurs between three and ten days after insemination. The present study found that egg-laying begins between six and ten days after initial insemination. Each egg is wrapped individually in the leaf of a water plant, which may help reduce predation risk and also provide the developing egg with oxygen (Diaz-Paniagua 1990). Females of the congeneric *T. marmoratus pygmaeus* showed preferences for particular weed species in one study (Diaz-Paniagua 1989) and smooth newt females seem to prefer flexible, fairly broad (~10mm) leaves in which to wrap their eggs (pers.obs.).

The eggs hatch after about twenty days (Bell & Lawton 1975). The larvae feed in the pond and grow rapidly until they reach metamorphosis. This occurs in late summer or early autumn and the timing depends on various factors. These include temperature, food supply and density of individuals present in the pond (Baker 1990a). The metamorphs leave the pond and remain terrestrial as efts until they are

sexually mature. Maturity is reached between three and seven years of age according to Bell (1977) but there is some doubt about the validity of the method he used to age individuals (Verrell & Halliday 1986). Females seem to mature later than males, perhaps in order to attain a greater body size at maturity, as female fecundity has been reported to be related to body size (Verrell & Francillon 1986; Baker 1990a).

1.1.3 Emigration and the Terrestrial Phase

Although the reports from different studies show a certain amount of variation, the adults appear to leave the pond from around June to August (Harrison et al 1983; Griffiths 1984; Verrell & Halliday 1985; Verrell & McCabe 1988). The emigrating newts continue their terrestrial life, feeding and maturing their gametes (Verrell et al 1986). From the study by Verrell et al (1986) it appeared that both sexes have a dissociated breeding cycle, where gonadal activity and maturation of gametes do not occur at the same time as mating behaviour (Crews 1987). Crews (1987) suggested that this kind of breeding pattern occurs in species where there is a regular but narrow window of opportunity for breeding, which is indeed the case for smooth newts. Thus the male and female mature their gametes over the terrestrial phase of their annual cycle and arrive at the pond in the following spring with their complete supply of eggs and sperm for that season.

The number of gametes that each sex will have been able to mature may well be determined by their feeding success during the terrestrial phase. The fact that the gametes are limited over the season will have a feedback to the mating strategy employed by each sex. Females might be expected to be choosy about which male they mate with, but males might also be expected to be somewhat discerning if their sperm supplies are limited. This issue will be discussed further in Chapter 6.

It is evident that our lack of knowledge regarding the terrestrial phase is a serious handicap to further research. Recent work done on *T. cristatus* by Baker (1992) offered the exciting suggestion that food intake during the terrestrial phase (measured as an index of body condition on arrival at the pond) was correlated with

the later development of a male's crest in the aquatic phase. Since the crest is regarded as a potentially sexually selected character (see Section 1.3.5 and Chapter 6) this has important implications with respect to Andersson's condition-dependent hypothesis of sexual selection (Andersson 1982, 1986). The hypothesis that crest height is correlated with male condition (and display performance) has recently been tested (Halliday, Waights & Hosie, in prep.). It is sufficient here to acknowledge that much more work needs to be done on the terrestrial phase. It may be easier to concentrate on fine details of the courtship behaviour that we know so much more about and dismiss terrestrial studies as too difficult. However many workers in North America have devised methods and approaches for studying terrestrial salamanders in the field (e.g. Gergits 1982, Gergits & Jaeger 1990; Hairston 1987; Jaeger 1986, 1990). In addition, Waights (in prep.) has developed laboratory techniques for keeping terrestrial adults and juveniles (*T. vulgaris* and *T. montandoni*). Hopefully in the future these field and laboratory methods may be integrated to investigate the activities of newts in the two-thirds of their life that has not really been considered so far.

1.2 The Sexual Behaviour of the Smooth Newt

Extensive research has been carried out on several aspects of the courtship behaviour of this species. These include a detailed description (Halliday 1974), analyses of factors affecting male behaviour (e.g. female behaviour, Halliday 1975; spermatophore supply, Halliday 1976; oxygen supply, Halliday & Sweatman 1976, Halliday 1977a), the effects of competition between males (Verrell 1984a) and the study of a breeding population in the field (Verrell & Halliday 1985). There is only scattered information about the female's role in courtship and her reproductive behaviour. This is reviewed in Chapter 5. Here, the courtship is briefly described, with an emphasis on the female's behaviour.

1.2.1 Courtship

Figure 1.1 shows an outline of the courtship. The female sometimes initiates a courtship encounter but usually the male approaches the female. They may turn their snouts towards each other's bodies and may touch the head, belly or cloaca. This is called a *sniff* (see Marquenie {1950} for earlier description, Halliday 1974) and a movement of the buccal surface can sometimes be observed at this time. The female usually shows less interest than the male. The male then attempts to *move-to-the-front* (Halliday 1974) by swimming swiftly alongside the female and jumping ahead of her, his body roughly perpendicular to hers. This stops the female from moving forward (at least momentarily) and the male tries to begin his *static display* (Halliday 1974). The female often runs or swims away and the male pursues her, trying repeatedly to *move-to-the-front*. If she eventually stands still, the male can then begin his static display.

Static display consists of variable amounts of three basic display behaviour patterns, fan, whip and wave (Halliday 1974). The male continues to display to the female, occasionally shifting his position to display on the other side of his body. He invariably tries to regain his display position if the female moves away. If the female is, or has become, responsive, she will approach the male, usually taking just a single step forward at first. The male usually responds to this with a whip and, if the female continues to approach, he starts to move backwards (still facing the female) in *retreat display* (Halliday 1974). In this display the male continues to whip, fan and wave (tending to employ different proportions of these elements than are used in static display {Halliday 1975}) but, at the same time, he is backing off from the advancing female. If she stops advancing, the male stops moving backwards.

If the female continues to approach the male, he eventually turns away from the female in *creep*, beginning the *spermatophore transfer phase* (Halliday 1974). The male is now moving forwards, with the female following him, both of them oriented in a straight line. He stops and performs a *quiver* movement (Halliday 1974). The advancing female bumps into the male's quivering tail, touching it with her snout.

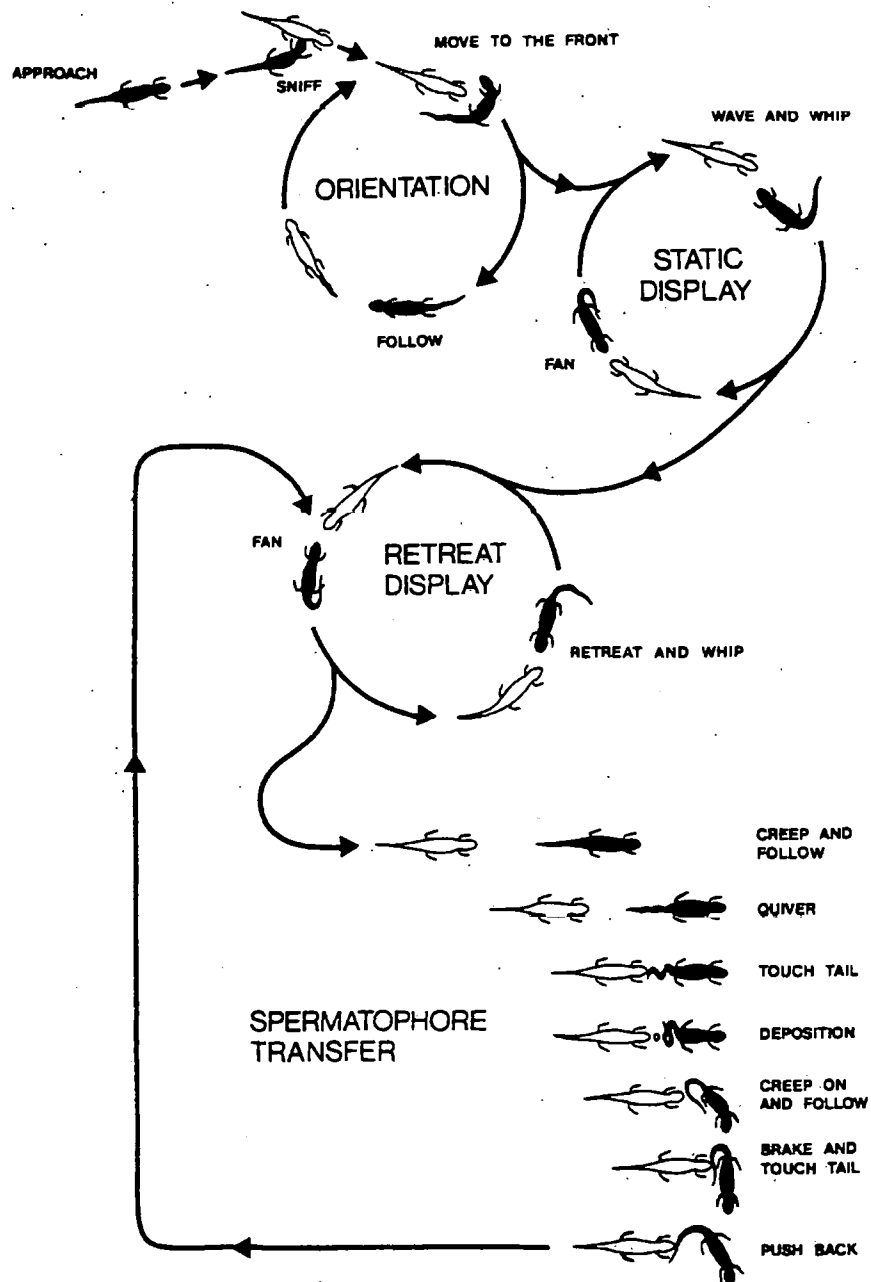


Fig. 1.1 Diagrammatic summary of the sexual behaviour sequence in the Smooth Newt. The male is in black. From Halliday (1974).

This *tail-touch* (Halliday 1974; 1975) by the female elicits spermatophore deposition by the male.

As the male concertinas and lifts up his tail to deposit the spermatophore, the female follows the movement of his tail with her snout, keeping it close to his cloaca. The male *creeps on* into *brake* position (Halliday 1974), turning through 90°. The female appears to follow the male's tail as it moves around into *wiggle* (Roberts, pers. comm.) as he turns into brake. The advancing female is stopped, by the positioning of the male, with her cloaca over the region in which the spermatophore was deposited. Although it has been suggested that the female is oblivious to the presence of the spermatophore (Halliday 1974), on some occasions I have seen the female dip her cloacal region over the spermatophore, or, at least the area in which it was deposited (see also Tinbergen & Ter Pelkwijk 1938). The sperm mass, situated on top of the spermatophore may be picked up by the papillae of the female's everted cloaca as she passes over it. The base of the spermatophore is left on the substrate and is not picked up (as was suggested by Halliday 1974).

The male may perform *push back* (Halliday 1974) movements after he has moved into brake and the female continues to approach him. Her snout contacts his folded tail in brake and this has been referred to as the second tail touch (Halliday 1974). It has been suggested that push back may serve to increase the chances of the female picking up the sperm mass (Halliday 1974, 1990) but evidence for this is slight. Halliday (1974) never observed a push back effect pick up but it has been noted once by Raxworthy (1989) and once in this study. From personal observation it seems likely that it could also function as a 'test' by the male to check how responsive the female is, or, to prevent her advancing too rapidly when he needs to replenish his supply of spermatophore components (Verrell {1986a} suggested that spermatophore production may limit male mating capacity).

After a few moments, the male begins to retreat in front of the female once more and, if she is still responsive, they go through the spermatophore transfer phase

again. This may be repeated up to six times in free courtship (pers. obs.) but it is more usual for around three depositions to occur.

Courtship is usually brought to an end by the female, who walks or swims away from the displaying male. On some occasions though, the male may terminate the encounter. This usually only occurs if the female is extremely responsive and the male has already deposited a number of spermatophores. While the female continues to approach, the male stops displaying and turns to sniff at the belly and/or cloaca of the female. Sometimes he resumes courtship but at other times he will stand still for some moments or go up to the surface for air. During this time the female will usually walk away and show no further interest in the male.

This section has described the simple situation of one individual of each sex courting together in an aquarium. The social situation in a pond may be very different (Verrell 1984a, 1986a; Verrell & McCabe 1988) and this must be borne in mind when discussing the results of experiments based on the simple dyadic situation, as pointed out by Halliday (1990).

1.3 Approaches

This project has embraced a number of approaches that need some clarification. Firstly, a female oriented viewpoint has been adopted, not simply because it is female behaviour that has been studied, but also to emphasize the importance of the female role in behaviour generally, which has been somewhat neglected (e.g. see Wasser 1983). This is discussed in Section 1.3.1. Secondly, the project has looked at both motivation and mate choice. Traditionally, work on motivation is regarded as a causal approach to the study of behaviour, trying to look at the mechanisms that underlie behaviour. The study of mate choice is seen as an essentially functional approach to behaviour, although some work has been directed at the role of hormones (see Hutchinson & Hutchinson 1983; Keverne 1983). The different approaches to the study of animal behaviour central to this study are considered below (sections 1.3.2 and 1.3.3), and then the concepts of motivation and

mate choice are discussed (sections 1.3.4 and 1.3.5), to provide a context for the study that follows.

1.3.1 The Female Role and Female Behaviour

The activities of females have received comparatively little attention in most fields of animal behaviour, including sexual selection. Sexual selection theory is obviously concerned with both males and females but empirical studies have dealt almost exclusively with males (see Blaffer-Hrdy & Williams 1983, for a review). This is unfortunate because, although female choice is of central importance to sexual selection, and is often invoked as a major selective force, it is rarely studied from the female's point of view. The actual courtship and mating behaviour of females and how this might vary over time has received even less attention.

Reviews of European and New World salamander courtship behaviour illustrate this point well. They are heavily biased towards the behaviour of the male and how it may have evolved (e.g. Halliday 1977b, 1990; Verrell 1989). Studies and discussions of females tend to focus on clutch sizes, egg-laying behaviour and parental care (e.g. Salthe & Mecham 1974; Houck 1977; Tilley 1977). The emphasis is thus on life history factors rather than the female's role in mating; the female is generally seen as a 'resource' for which males must compete (e.g. Krebs & Davies 1987).

It has already been mentioned that males will tend to invest less in each of their gametes than females do in each of theirs (Trivers 1972). Investment by the female may take different forms. In urodeles for example, females of one species, e.g. *Ambystoma talpoideum* (Salthe & Mecham 1974) may lay hundreds of eggs in clumps with no parental care, while others, e.g. *Plethodon cinereus* (Arnold 1977) lay around ten eggs and actively guard them until they hatch. These different patterns of investment and trade-off between egg number and investment, considered in their ecological and life history contexts, may have a very important influence on whether female choice exists in a particular species; and if it does, how important it has been in

shaping that species' courtship. The obvious importance of the female's role in courtship and mating is belied by the paucity of information available.

Verrell (1989) suggests that 'the major adaptive variation in the sexual behaviour of urodeles appears to have been in mechanisms employed by males to render females sexually responsive'. This overtly male-oriented viewpoint suggests that female responsiveness is a simple state that a male needs to arouse in a female, in order for insemination to proceed, and does not entertain the possibility of variation in female responsiveness. The consideration of female responsiveness in the literature requires some discussion at this point.

While it has been shown that females of some species require exposure to males in order for them to fully come into reproductive condition (e.g. Moore et al 1979; Crews 1980; Lindzey & Crews 1988; Widowski et al 1991), this, strictly speaking, has more to do with females' capacity for responsiveness than with their responsiveness *per se*. It is also well recognised that male behaviour over a courtship encounter can alter a female's responsiveness (Verrell 1982; Teyssedre & Halliday 1986; Houck & Reagan 1990).

However, there is a general idea that at the beginning of a courtship encounter females are unresponsive, whereas males are ready to mate (Halliday 1983a; in press). Whilst this may seem to be the case, it may partly be that the male simply appears to be more active; the female may be ready to mate but not exhibit any behaviour that readily indicates the situation. In any case, it is perhaps as potentially misleading to assume that female *un*responsiveness is a simple state as it is to assume that female responsiveness may be.

Simple recognition of the fact that female responsiveness may vary over time and that females play a dynamic (though not necessarily active) role in courtship and mating is important as it promotes a more balanced and thus much clearer understanding of courtship as a complex interaction between two partners, and its evolution as such. However, it also has very important implications for mate choice studies. Halliday (1983a) emphasised the important general point that if a female does

not mate with a male, it may be because she has 'chosen' not to according to some criterion pertaining to the male, or it may be because she is not sexually motivated and would not mate with *any* male. Unless the female's sexual motivation at the time of mating is known, conclusions about female choice are difficult to make.

Another consideration with regard to mate choice studies is that female responsiveness may be linked to the ability of females to make discriminations between males. If responsiveness varies over time and under different circumstances so may the criteria upon which mate choice 'decisions' are based. In addition, the extent to which mate choice is important may vary over the breeding season. Much of the variation observed in studies of mate choice may be explained by a better understanding of female responsiveness, and the variations observed in courtship 'can only be fully understood when we understand the dynamics of female receptivity' (Halliday 1990).

Returning to the question of whether females are generally unresponsive, many studies in a variety of taxa indicate directly or, more usually, indirectly that female responsiveness can indeed vary (e.g. Steel 1980, hamsters, *Mesocricetus auratus*; Rutowski 1980, butterflies; Gwynne 1985, crickets; Verrell 1988, *T. vulgaris*; Pitnick 1991, *Drosophila melanogaster*; Propper 1991, Californian newts, *Taricha granulosa*). It is hoped that this thesis will demonstrate that, at least in smooth newts, female responsiveness is a somewhat complex phenomenon. The previous smooth newt studies which have dealt with or have referred to female behaviour are reviewed in Chapter 5.

1.3.2 Approaches to Studying Animal Behaviour

This study embraces both causal and functional approaches to animal behaviour. Thus the state of these two approaches in present day ethology will be briefly reviewed.

Tinbergen (1963) clarified the four now familiar approaches to the study of animal behaviour; causal, functional, developmental and evolutionary. These still

provide the basic framework for work done today but emphases have shifted with changing fashions in biology. Of these four approaches, the study of causation (also referred to as proximate causation) and function (also called ultimate causation) have engendered perhaps the largest amount of research in animal behaviour, although important work has also been conducted using the developmental and evolutionary approaches (e.g. Slater 1985).

Causal studies generally investigate the underlying mechanisms that generate a particular behavioural action or coherent behaviour pattern (e.g. Huntingford 1984; Krebs & Davies 1987; Slater 1979, 1985). Functional explanations consider how adaptive behaviour is and why natural selection has led to and maintains that particular behaviour (e.g. Huntingford 1984; Krebs & Davies 1987; Slater 1979, 1985).

A glance at the contents page of a textbook on animal behaviour from the early 1970s (e.g. Hinde 1970; Alcock 1975) indicates the extent to which early ethology was focussed on causation. As studies diversified, behavioural ecology, with the functional approach at its heart, has grown up and blossomed in the last fifteen years (e.g. Krebs & Davies 1978, 1984, 1987). Slater (1979) feared that as studies had become more detailed the two approaches had tended to drift apart, even though he considered much of the best work in this area had involved looking at problems from both viewpoints.

Ten years on, a number of authors feel that these approaches have diverged and that function has been overemphasised; they propose that ethologists need now to emphasise studies that combine the different approaches, as advocated by Tinbergen (1963) himself (Dawkins 1989; McFarland 1989; Davies 1991; Halliday in press; Huntingford 1991; Krebs & Davies 1991).

1.3.3 The Causal Approach

This will be discussed in a little more detail as it forms the basis of much of the work described and, as it has received relatively little attention recently, it needs some clarification.

There are different levels of approach to the study of causation. At the most reductionist level is what has been called the 'hardware' approach (Huntingford 1984) where explanations of behaviour depend upon a knowledge of the actual physiological mechanisms involved. It is obvious that, at one level, all behaviour depends on the nervous system and physiology of the animal, but it has been argued that this can only ever give a partial picture (Manning 1979; Slater 1985).

At a different level, the 'software' or 'black box' approach deals with the whole animal rather than the physiological events inside it (Huntingford 1984; Slater 1985). Treating the animal as a black box, its behavioural output is studied in terms of behaviour, in response to changes in various inputs such as external stimuli (Slater 1985). This kind of study can enable behaviour to be predicted and can identify phenomena that require explanation at the physiological level. Huntingford (1984) and Slater (1985) argue that both approaches are important and that a combination of both seems most sensible. Slater (1985) feels that a complete knowledge of the causes of behaviour requires information about its organisation at the behavioural level and about the physiological mechanisms underlying it. He cites studies by Nottebohm (1984; Nottebohm et al 1976) on the neural basis of song in canaries (*Serinus canaria*) as an example of work that has made contributions at both levels; the detailed work by Ewert (1980) on prey catching in toads is another.

Very recent reports (Guildford & Dawkins 1987, 1991; Ryan 1990; Krebs & Davies 1991) have suggested that another level of causal investigation may be emerging in the study of behaviour; that is the study of animals' sensory and perceptual systems. Instead of beginning with the idea of studying the mechanism underlying some behaviour for its own sake, the work by Ryan (1990) has grown out of the desire to understand the sensory basis of female choice, ultimately to study sexual selection (see also Chapter 7). Guildford & Dawkins (1987) show that investigating the mechanism that predators use to hunt cryptic prey can alter traditional views on the adaptive significance of prey appearance. As Dawkins (1989) points out, and as is clear from Guildford & Dawkins (1991), the understanding of animal

behaviour would greatly benefit from the investigation of the perceptual worlds of animals (see also Chapter 7).

The causal approach taken in this project is essentially at the behavioural level. Little is known about the physiology of newts but their sexual behaviour has been studied in detail. This project aimed to investigate the factors that influence sexual behaviour by careful observation of individuals under different conditions. In doing this, information about the mechanisms that underlie the control of the behaviours can be deduced and any phenomena that lend themselves to more physiologically based study can be identified.

1.3.4 The Concept of Motivation

Tempting as it is to tiptoe around the edge of the muddy minefield of terminology that accompanies the study of motivation, it is necessary to at least attempt to make some sense of it. Much confusion has arisen and has persisted over decades about what exactly is meant by the term motivation. Ethology borrowed the term from psychology and brought its own shades of meaning to it, which are not always clear. Some ethological texts sidestep the issue and leave the reader to decide what is meant; others give a rather all-encompassing 'definition' which makes clearer what is meant but does not provide a really useful or useable definition. An example of a this very broad kind of definition is that given by Slater (1985); he describes motivation as 'the mechanisms leading animals to do what they do when they do it'. A slightly tighter definition is given by Halliday (1983b), where motivation 'encompasses a broad category of phenomena that have in common that they are dependent on mechanisms internal to the animal and that they are reversible'.

On the whole ethologists seem less concerned with trying to formulate strict definitions than getting on with studying the animal they are interested in. This may not be a problem if they are clear about how they view the concept of motivation, but to someone new reading about their work, it is not always obvious. This can generate and perpetuate confusion. However, ethologists are probably less aware of any

confusion than are psychologists since most discussion about motivation is confined to text books (e.g. Halliday 1983b; Huntingford 1984; McFarland 1985; Colgan 1989), as very little new ethological research has been directed at motivation in recent years. There is thus less immediate need for workable definitions and discussions of them.

Psychologists studying animal behaviour have for years tried to form careful definitions of motivation. Zucker (1983) discusses the many and varied definitions that exist and the extent to which different workers consider a concept of motivation to be useful. Some definitions resemble those more familiar to ethology (e.g. Hebb 1966: 'tendency of the whole animal to produce organised activity'; Mook 1987) but others strive for a stricter definition, as is evident from the discussion that follows.

Recently, Wise (1987) reviewed definitions of motivation from the 1920s to the present and concluded that an adequate definition still does not exist. He revisited Skinner's (1953) criticisms of definitions of motivation, taking issue with their mentalist connotations and their inherent circularity. He went on to devise a definition that he considers to be free of circularity as it has its basis firmly in physiology and thus also avoids the problems of mentalist terminology (Wise 1987). He defines a motivational state as 'a state of *selective modulation* of the effectiveness of classes of response-eliciting environmental or proprioceptive stimuli (or their central correlates), mediated by a central mechanism involving *hormonal* or *neruomodulatory* elements' (Wise' own italics).

Wise (1987) seems to run into problems however, because his definition is based on the premise that both motivated and non-motivated behaviours exist. He designates the Fixed Action Pattern (FAP, borrowed from ethology) as the unit of non-motivated behaviour and the Variable Action Pattern (VAP) as the unit of motivated behaviour. Blackburn & Pfaus (1988) take issue with this saying that FAPs are not as invariable as was once thought and that no real physiological distinction exists between these and VAPs. Wise (1987) states that if non-motivated behaviour does not exist then the need for a concept of motivation disappears.

In a similar vein, Kennedy (1987) traced the search for motivation that underlies Dethier's work on fly feeding. He considers that there is no need for the term motivation because Dethier found no dichotomy of behavioural mechanisms (the idea being that simple reflex behaviour is non-motivated and 'higher' modes of behaviour are motivated). Kennedy (1987) is also adamant that the continued use of the term motivation and other mentalist terminology leads to proximate and ultimate explanations for behaviour being confused. This is a problem that Tinbergen himself anticipated (1951) and an example of the misunderstanding that it can engender is discussed by Davies (1991). Mook (1987) succinctly summarises the controversy: 'one can define (motivation's) domain so broadly that it encompasses all of psychology, or so narrowly that it threatens to vanish entirely'.

So where does all this agonising over a definition leave us? The confusion seems to be centered around whether one feels that motivation is some entity or force present in an animal which generates behaviour, or whether one considers motivation to be merely a 'construct imposed by the observer to give order to his or her observations' Blackburn & Pfaus (1988). Dawkins (1986) advocates caution in the use of the term, considering it useful but easily misused. It is too easy to slip into loosely talking about different motivational systems as though there was no internal interaction between an animals' activities (e.g. see Colgan 1989; Pellis 1991).

I feel that, as long as the way in which the term is to be understood is carefully explained, and the behaviour under observation is carefully defined, the concept of motivation can still have a useful role in the study of animal behaviour. The usefulness of it to this study will be briefly considered in the final chapter.

1.3.5 The Concept of Mate Choice

The prominent dorsal crest of male newts was, along with peacocks' trains and brilliantly plumaged male birds of paradise, one of the examples of extreme sexual dimorphism that gave Darwin much cause for thought and led him (1871) to propose the concept of selection in relation to sex. He suggested there was a distinction

between intra-sexual selection (usually understood as competition between males for females) and inter-sexual selection (females choosing between different males). These ideas have resulted in much research in this area, field based and theoretical, and reviews of different aspects abound.

It seems somewhat excessive here to dryly re-review the area of sexual selection and mate choice; rather, identification of key areas of interest and fruitful research would seem more useful.

The concept of female choice has been controversial since work in this area began. At first it seemed a very plausible explanation for how sexual selection might have acted and still operates, but it has proved notoriously difficult to actually demonstrate. Female choice hypotheses centre on two basic ideas: Fisher's runaway hypothesis and the 'good genes' school of thought.

Firstly, Fisher (1930) formalised the idea that male ornaments (which intuitively might hinder survival) may have evolved simply because females prefer to mate with the most ornamented males. This would confer an advantage on their sons (in terms of being attractive to females) and on their daughters (the preference for ornament will be passed on) and thus the preference will be maintained. With regard to the problem of how this situation could have arisen, Lande (1981) and Kirkpatrick (1982) have shown mathematically that the male trait need not be initially advantageous as long as some degree of female preference for it exists in the population beforehand.

The second hypothesis is that females may choose mates on the basis of criteria that are indicators of viability and thus these indicate 'good genes' which will be passed on to the female's offspring. Andersson's (1982; 1986) condition-dependent hypothesis of sexual selection, and its relation to mate choice in newts, is considered in detail in Chapter 6.

Borgia (1987) reviews the role of female choice in models of sexual selection and Pomiankowski (1988) considers the evolution of female mate preferences for male genetic quality. Maynard Smith (1991) reappraises theories of sexual selection in the

light of recent research that suggests that 'handicaps' (Zahavi 1975) and 'honest signalling' may be more important than hitherto thought.

As well as these more general growth areas of research a number of specific pointers for fruitful new research are emerging. Halliday (1987) identified and elaborated on the importance of examining the physiological constraints on sexual behaviour in more detail, particularly that of males. He emphasised how important this is for understanding the determinants of male mating success and the benefits females might derive from making a choice. This is also true for females; very little is known about the physiological constraints (or indeed any constraints) on female sexual behaviour.

Earlier (Section 1.3.1) the point was made that closer study of female behaviour would encourage a better appreciation of female choice; some recent work begins to illustrate this. Sullivan (1990) discusses how females might assess males when the male's characters vary during the sampling period. A number of workers have recently found that choice appears to be based on multiple criteria in a complex way (e.g. Burley 1981; Zuk et al 1990) or that the criteria used for choice may differ in, for example, different years (e.g. Sullivan 1987; Reid & Weatherhead 1990). It may be that variability in male traits is more important than the traits themselves (Reid & Weatherhead 1990) or that female 'choices' are more complex than has been recognised so far. Either way, more research directed at female behaviour, particularly responsiveness, can only improve our understanding of female choice and sexual selection.

This study aimed primarily to examine female sexual responsiveness in smooth newts using both causal (Chapters 3 & 5) and functional (Chapter 4) approaches. This was complemented by an investigation into aspects of female choice (Chapter 6) and detailed consideration of the importance to the female of the different stimuli presented by the male during courtship (Chapter 7). It was hoped that this combination of approaches and perspectives might yield a better understanding of both

female sexual motivation and female choice, than could be achieved with a single approach (see Chapter 8).

CHAPTER 2

General Methods

2.1 Sources of Animals

Two ponds in Milton Keynes provided the majority of animals required for the project. Great Linford housing estate boasts a small (~12m diameter, <1m deep) pond as the major feature of a roundabout. The position of the pond means that individuals can be collected easily at the start of the season, by simply picking them up off the road as they migrate to the pond on wet warm evenings. These individuals have not yet reached the pond and will have had no chance to mate. These are therefore ideal for use in experiments where unmated animals are required. This pond has a rubble substrate because it was partially filled-in by the local council to reduce the potential danger for children on the estate. This provides countless refugia for the newts when they are in the pond and it is almost impossible to catch any after they have migrated to the water.

When supplies of fully aquatic newts were needed they were obtained from a pond in the Conniburrow housing estate. The pond is around 10m in diameter and less than 80cm deep, with a flat mud substrate which is littered with numerous items of human rubbish. The water is generally clear and the newts can be caught after dark by dipping with a handnet. Around approximately half of the pond's perimeter is a wooden slatted wall which the newts find very attractive; it provides numerous crevices for them to hide in. Conveniently, they can sometimes be coaxed out of them, which proves an easy method of capture.

Other newts were collected in small numbers from three further sites: a large garden pond (~15m diameter) in Oxford, where newts were caught in traps; a small farm pond (~6m diameter) in Soulbury, Buckinghamshire and a disused (now disappeared) swimming pool in Stony Stratford, Buckinghamshire. Newts were caught by dipping with nets in the latter two ponds.

Newts were returned to the pond from which they were captured as soon as possible after being used in tests. In addition, any eggs laid over the season were maintained and allowed to hatch. The resulting larvae were returned to the pond with the adults in an attempt to reduce any impact that repeated collecting might have had on the population in the long term.

2.2 Maintenance of Animals

2.2.1 Preliminary study

For the preliminary study, carried out in 1988, animals were kept in a temperature-controlled room with no source of natural light. The temperature was maintained at 14°C and a fixed artificial light/dark schedule was in operation to mimic the light/dark cycle.

The females under study were kept individually in small tanks (20x25x40cm) while the males were kept together, according to location, in larger tanks (30x30x90cm). The small tanks housing the females were filled with natural pond water and various pond weeds to try and make them as 'natural' as possible. This was an attempt to eliminate previous problems encountered when trying to keep newts in these tanks over a long period of time (females go quickly out of condition and will not egg-lay; Baker, pers. comm.).

Newts were fed regularly on *Tubifex* worms, sometimes *Daphnia*, and were also occasionally given chopped earthworms.

2.2.2 Further studies - improvements in general maintenance

For all the remaining work (1989 and 1990) newts were maintained in semi-natural conditions at an outdoor site. The site has a small shed with tanks placed on racking inside. The shed is unheated unless observations are being made when a small fan heater is switched on. In addition, a small heater comes on if the temperature falls below 4°C to stop the shed from freezing.

Outside the shed are large plastic tubs (2.5m diameter, 1m deep), originally designed for keeping fish. The tubs are supported above the ground and have lagging around them. They have covers designed to keep out 85% of the sunlight and these also afford some protection against frost in the colder months. Thus the site is exposed to natural environmental fluctuations with some protection measures in case of extremes of temperature. Some tanks were maintained in the shed while others were placed in the tubs, surrounded by water to buffer them against extreme temperatures (see Fig. 2.1).

Animals kept in the temperature-controlled, fixed light-schedule room in 1988 tended to go out of breeding condition quickly (within a couple of weeks) and none of the females laid eggs under these conditions. The new site meant that animals were exposed to natural fluctuations in light, temperature, daylength *etc.* While this introduces sources of variation that were previously controlled for, it does mean that interesting information can be obtained about the effects of these variables on the newts' behaviour. Most importantly, however, the newts flourished in this environment. Both sexes remained in breeding condition for longer than they did in the indoor room and the females laid eggs under these conditions.

2.2.3 Further studies - maintenance of animals

Females for some studies in 1989 and 1990 were collected on migration to their pond. Past experience suggested that animals collected before reaching the pond, and put immediately into water in the laboratory did not always achieve breeding condition very satisfactorily or did not maintain condition very well. Newts need to undergo various changes to their skin, respiratory system *etc.* before they can fully adjust to an aquatic environment (e.g. see Halliday 1972). In an attempt to alleviate this problem the newts were put into 'migration' tanks after collection (see Fig. 2.2) to give them time to become fully aquatic at their own pace.

Females kept individually were housed inside the shed in large tanks (30x45x60cm) filled with aged tap water and containing pieces of brick and large

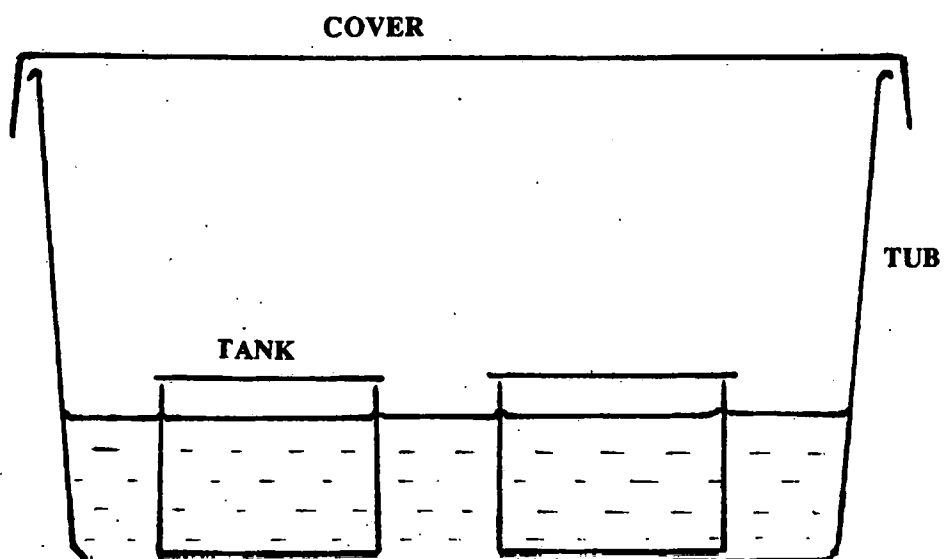


Fig. 2.1 Set up of outside tubs showing the arrangement of tanks within them. The tub is raised above the ground and has lagging around it. (See text for details).

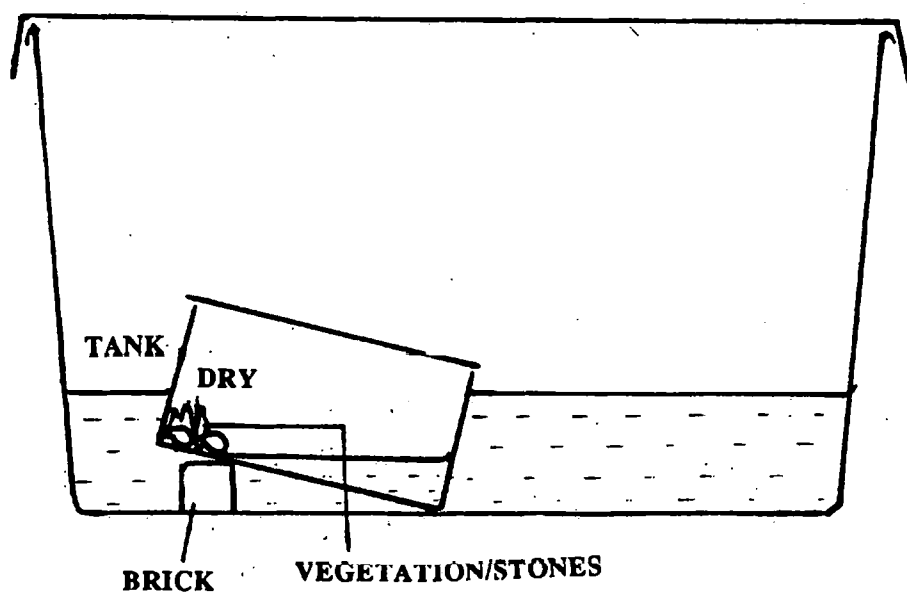


Fig. 2.2 Arrangement of 'migration' tanks set up in outside tubs. (See text for details).

pebbles for refuge, on a substrate of gravel. A small quantity of natural weed was put into each tank but most of the 'weed' was made from strips of biodegradeable green polythene. This was provided so that females could deposit their eggs on it and there they could then be counted and collected. The colour green was chosen to eliminate potential problems if colour is important to females in their choice of plant species for this purpose. This biodegradeable polythene does not float very well and so stays in the body of the water better than other types without having to be weighted. This kind of polythene is also more flexible than many others, which may also be an important factor for females in deciding where to lay their eggs (Diaz-Paniagua 1986).

Other newts were kept in tanks (30x45x60cm or 30x45x120cm, depending on numbers of individuals) either in the shed or, in most cases, in the tubs outside where they would be surrounded by water. The sexes and the different populations were maintained separately.

2.3 Experimental Procedures

The majority of experiments were carried out in the following way. A single female and a single male were placed in an observation tank (30x45x60cm), one each side of an opaque partition, to keep them separate. They were allowed to settle for at least five minutes. The partition was then removed and they were allowed to court if they wished. For most experiments straightforward observations of the freely courting pairs were made, noting particular behaviour patterns performed by the male and/or female, as required by the experiment. Data were recorded on paper and/or audio tape, speaking into a hand-held tape recorder (SONY Voice Operated Recording Cassette-Corder TCM-23V).

The pair remained together for a pre-determined length of time, according to the experiment. Each newt was either individually identifiable or its various parameters were measured after the observations had been completed.

The preliminary study (1988) was carried out in the laboratory where the temperature of the water in the observation tanks varied between 19°C and 21°C. The

further studies (1989 and 1990) were carried out in the outside shed. The water in the observation tanks here varied between 5°C and 20°C according to the outside temperature over the season.

Details of specific methods relevant to particular experiments are discussed in the appropriate chapters.

CHAPTER 3

Preliminary Study of Female Sexual Motivation

3.1 Introduction

3.1.1 General Introduction

The concept of motivation was reviewed in Chapter 1 (Section 1.3.4). In this study, 'motivation' is simply a short-hand way of referring to the internal state of an individual, which is the result of the interaction of a number of factors (internal and external). Thus for example, in a female newt, internal physiological factors and external factors (e.g. male display, time of day, etc.) could interact such that she mates immediately with a male she encounters: i.e. she has a high sexual motivation.

This study has taken a 'black box' approach to studying sexual motivation (see Section 1.3.3). Females were treated as black boxes and their behavioural output was observed and monitored in response to changes in external stimuli. The behaviour that was actually studied is referred to as female sexual responsiveness, or receptivity, to an individual of the opposite sex.

It has already been mentioned (Section 1.3.1) that studying female responsiveness is crucial to a fuller understanding of female choice. Although the phenomenon should be considered for all species being studied, for those with a short, intensive mating period it presents fewer difficulties. For example, the guppy *Poecilia reticulata*, has a brief mating period of a few days (Houde 1987). During this short time most individuals are initially relatively sexually highly motivated and mate choice is relatively easy to study, as each individual's motivation is probably roughly similar.

Smooth newts, in contrast, have an extended breeding season of around three months, over which time an individual's sexual motivation may vary considerably. In an earlier experiment, in which the responses of inseminated female smooth newts to further exposure to males were examined, Verrell (1984b) showed that there was considerable

variation in how responsive to males females could be in the twenty days following insemination. Although nearly half (12/25) remained unresponsive for that time, six showed a 'weak response' (Verrell 1984b) (they responded to the initial stages of courtship), five elicited spermatophore deposition but did not pick up sperm and two picked up sperm from at least one of the spermatophores deposited.

Thus it seems that the sexual motivation of female newts can be variable and unpredictable, at least over twenty days. As well as the implications already discussed, this is also particularly pertinent to the question of sperm competition in this species. Since the female can store sperm in her spermatheca (Halliday & Verrell 1984) and can pick up sperm from more than one male (Verrell 1984a, 1984b; pers. obs.), it certainly seems likely that sperm competition may occur (Halliday & Verrell 1984; Verrell 1984b). The importance of sperm competition as a form of sexual selection (Parker 1970) deserves consideration in this species; it is discussed in more depth in relation to multiple mating in Chapter 4.

3.1.1. Studying and Measuring Motivation

It would seem relatively easy to assess sexual motivation in *T. vulgaris* males, since they perform a complex, stereotyped sequence of courtship display behaviour patterns, each of which is detectable and measurable (in terms of frequency or duration). Females present more difficulty since their sexual behaviour is relatively inactive, and even at the climax of the courtship they appear to be simply walking forwards. In this study females were observed intensively to try to identify some pointers in their apparently passive behaviour which might reliably indicate the level of their sexual motivation.

Female sexual motivation may vary in the long and the short term, so two distinctly different timescales for this variation were explored in this study. Firstly, the aim was to chart female sexual motivation over several weeks, to look for any underlying

patterns in responsiveness in the long term. Secondly, the detailed changes in responsiveness over single courtship encounters were studied, to see how male courtship affects female motivation. In this way, a detailed picture of how female sexual motivation varies in the long and the short term can be built up, and some idea of how these variations relate to each other can be obtained. Simple models that suggest how long term and short term variations may relate to each other are discussed below.

Halliday (in press) has proposed a causal model of female choice that takes into account variations in female responsiveness. The parts of the model that deal with male 'potency' (Halliday in press) are not dealt with here; only the part that deals with female receptivity will be discussed. The model assumes that there is a state within a female, called her receptivity, which varies over time, and that a female will mate when her receptivity exceeds a certain threshold value.

The model suggests that receptivity is initially at a low level at the beginning of the season but increases spontaneously with time. This may be due to external factors such as day-length, temperature, etc., interacting with internal factors such as hormones (see Fig. 3.1a).

It is known from previous work (Teyssedre & Halliday 1986) that male courtship in *T. vulgaris* can increase a female's responsiveness. Thus, in addition to the 'background' level of receptivity, male courtship can act to further increase the female's motivation. What is not known is whether this effect of male courtship is cumulative, or, whether courtship has no further effect upon the female after the encounter has ended. Male courtship behaviour could affect the female's motivation in different ways. Two possibilities are entertained in this model:

- i) the effect of a bout of courtship may last no longer than a single encounter (this is represented diagrammatically in Fig. 3.1b);
- ii) male courtship may have a more lasting, cumulative effect, each encounter building on the effect of the previous one (this is represented in Fig. 3.1c).

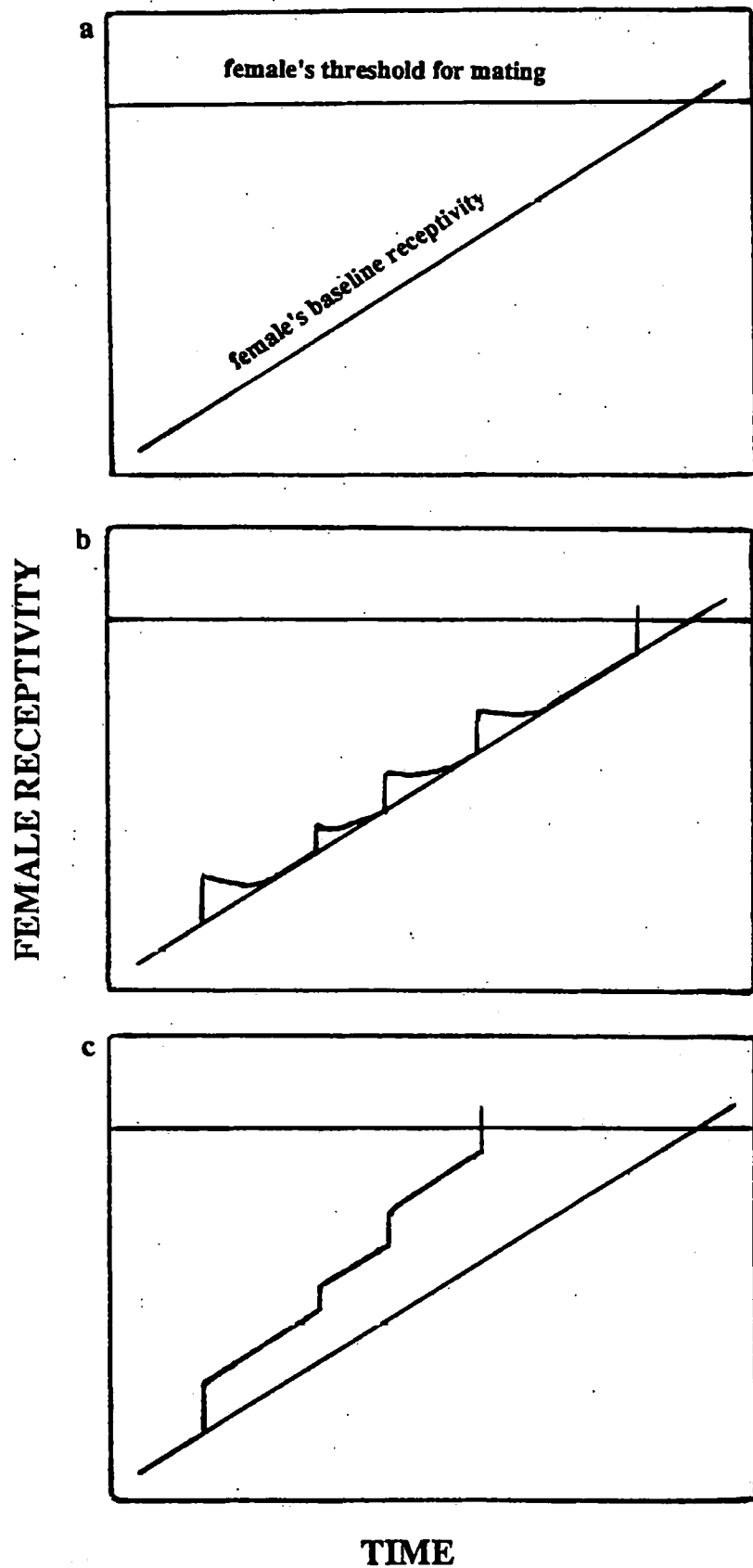


Fig. 3.1 Causal model of female choice taking into account variation in female responsiveness. (See text for details of a,b,c). Modified from Halliday (in press).

3.2 Aims

The present experiment takes an observational approach, focussing on individuals and monitoring them over time, in a longitudinal fashion (Martin and Bateson 1986). The experiment was devised to study two aspects of female sexual motivation:

i) how an individual's sexual responsiveness changes over a long timescale (i.e. weeks) and whether any underlying pattern of change emerges: that is, to see whether Fig. 3.1a correctly depicts the natural situation;

ii) the effect of male courtship on female motivation and whether this courtship has a short term or a cumulative effect: that is, to see whether either of the hypotheses shown in Fig. 3.1b and Fig. 3.1c represent the true picture.

Thus the experiment was designed so that each individual female received a standard amount of courtship from the male in each test, and the tests took place at set intervals.

3.3 Materials and Methods

3.3.1 Newts

Male newts were collected around 10th/11th April 1988 from ponds in Soulbury, Stony Stratford and Oxford. They were housed in large tanks (see Section 2.2.1) and represented a 'pool' of sexually active males for use when testing the females.

Females were collected from the Soulbury and Oxford ponds. Eight females were housed individually as described in Section 2.2.1. Although this may seem a small sample size, preliminary observations showed that eight was the most practicable number that could be studied in such a detailed fashion, without large time intervals between each of the tests.

3.3.2 Testing

After collection, all the animals were left to settle for about a week before testing began. A test consisted of placing one male and one female in a tank and making observations as they were allowed to court freely (as described in Section 2.3). All tests were carried out between 21/4/88 and 12/5/88 (a.m.) in aquaria in the laboratory (see Section 2.3). Each female was tested once every two days (except for the final test which took place after a break of two days). The testing was terminated after the eighth test as one female had died after the seventh test and others appeared to be becoming terrestrial.

A number of preliminary observations were made of freely courting pairs (using newts that were not part of the experiment). This was done for two reasons, firstly to determine any aspects of the females' behaviour that might be useful to note in the tests, and, secondly, to determine the criteria for the 'standard' amounts of male courtship to be used in the actual tests.

3.3.3 Criteria for Male Courtship

The criteria for the courtship tests are detailed below.

1. The standard amount of male courtship a female received in a test was five 'courtship initiations', which can be defined as any attempt at courtship by the male that includes at least one of the components of static display (i.e. move-to-the-front, wave, whip or fan; as defined by Halliday (1974) and described in Section 1.2). On some occasions this was not possible if the female fled from the male's every approach; though of course this response by the female still provides interesting information.

2. If either sex (but usually the male) swam to the surface for air, the pair were allowed to come together again and the resumption of courtship was treated as a continuation of the same courtship initiation. Sometimes a new move-to-the-front did not occur as the male came down from the surface to his former display position.

Sometimes the males were a little reluctant to court, especially in the later tests, and if the females were themselves reluctant. In these cases the male was 'encouraged'

with an anaesthetised female in a strait-jacket making positive movements towards the male (see Halliday 1975). This problem and the validity of these 'encouragements' are discussed in a later section (3.5).

3.3.4 Female Behaviour Patterns

The female behaviour patterns noted in the preliminary observations usually took the form of an apparent response to a male's courtship. The behaviour of the female towards the male when the pair first encountered each other was recorded as the 'initial response'. This, and the responses to each of the five courtship initiations, were categorised as either negative, neutral or positive, defined as follows:

Negative: female swims quickly away when the male approaches or continues walking straight past the male when he attempts to initiate courtship. The female appears completely unaware of the male as he attempts courtship and does not stop to sniff him or sniff when facing in his direction.

Neutral: female remains still in response to the male's courtship initiation but does not approach him. There may be some sniffing by the female in the male's direction. After allowing the male to display for a certain time the female ends the courtship initiation by walking or swimming away.

Positive: Female stands still in response to the male's display (usually also sniffing) for a few seconds, then actually walks towards the male. The displaying male goes into retreat display (Halliday 1974, Section 1.2) and courtship continues to the spermatophore transfer stage (Halliday 1974, Section 1.2).

The males were measured at the end of each test. Snout-vent length (SVL), overall length (OAL) and tail height (TH) were measured to look at aspects of male morphology. Females were measured (SVL and OAL) at the end of all the testing. There turned out to be so few actual inseminations that any kind of statistical comparison

between males that successfully inseminated females and those that did not was not possible.

3.4 Results and Discussion

3.4.1 Inseminations and Spermatophores

Of the eight females tested, only four became inseminated, and each was inseminated on only a single occasion. This means that insemination occurred in 6.25% (4/64) of encounters. Female 2 became inseminated in the seventh test, female 5 in test one, female 7 in test two and female 8 in the fourth test. In each of these successful courtships (i.e. sperm was put down and picked up) three spermatophores were deposited. Females 2 and 7 picked up spermatophores 1 and 2, female 5 picked up number 2 only and female 8 picked up numbers 2 and 3. Other spermatophores were picked up on other parts of the body or were missed altogether.

3.4.2 Response Scores

As defined earlier, the female's behavioural response to the male's behaviour was recorded as either negative, neutral or positive. This was recorded for the initial response to the male and in response to each of his five courtship initiations. These categories were devised to try and obtain some kind of analysis of the behaviour patterns observed in the female, and of the changes in her sexual motivation. Each category was given a 'response score'. A negative response from the female scored 1 point, a neutral response 2 points and a positive response 3 points. If in a test a female floated around the tank or began shedding her skin, she was unable to make a proper response to the male that could be categorised as either negative, neutral or positive, and was given a score of zero.

In this way a score for the female's initial response to the male was obtained for each test. It was hoped that this initial response might give an indication of the female's

motivational state at the beginning of the test and that her later responses to the male's courtship initiations might reflect this initial response. Similarly, five response scores, from each of the courtship initiations, were obtained for each female in each test. These five response scores were added together to produce a 'motivation score' for each female in each test. This motivation score was taken as a measure of the female's sexual motivation at the time of the test.

Thus, using these response scores, the motivation of an individual could be monitored (albeit rather crudely) over the timescale of a single test. Also, the motivation scores could be plotted for all the tests, to generate a picture of how a female's motivation varied over a longer timescale. This latter case will be discussed first.

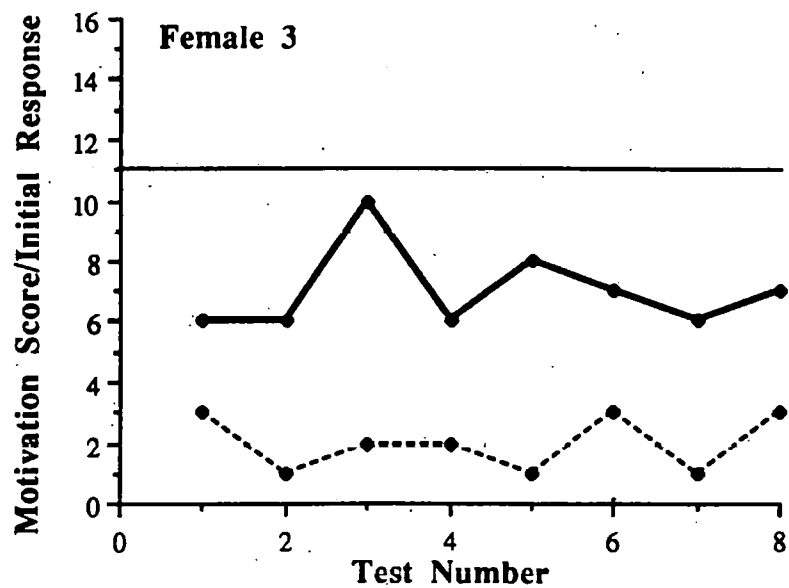
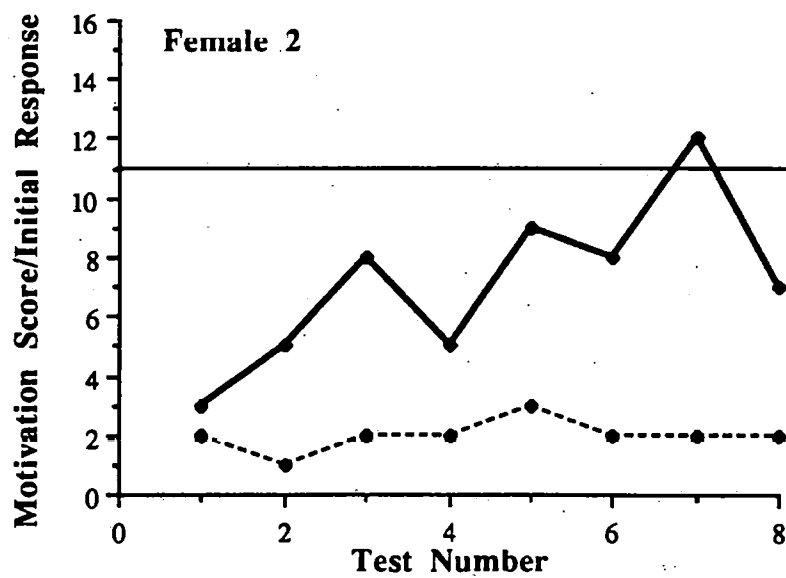
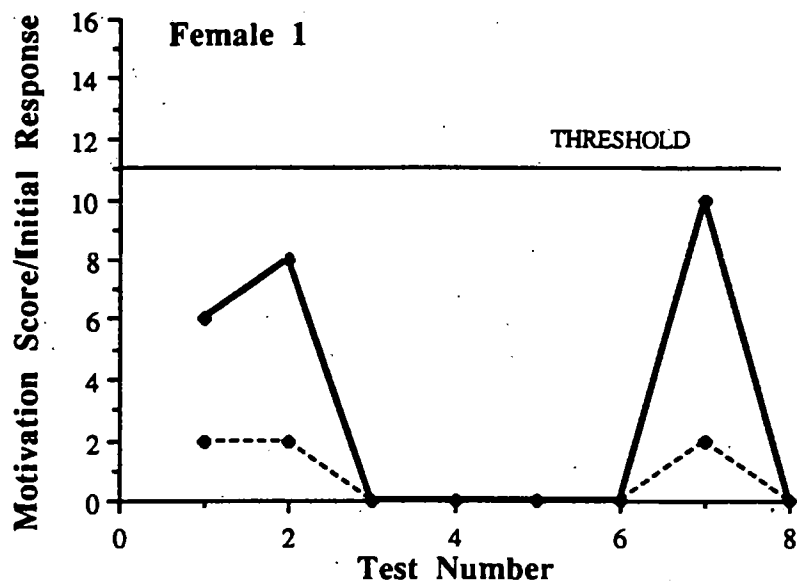
3.4.3 Female Sexual Motivation Over a Long Timescale

The motivation scores for each test were plotted together to obtain a profile of how an individual female's motivation varied over the three weeks of tests. Figure 3.2 shows the motivation profiles for the eight females over all the tests.

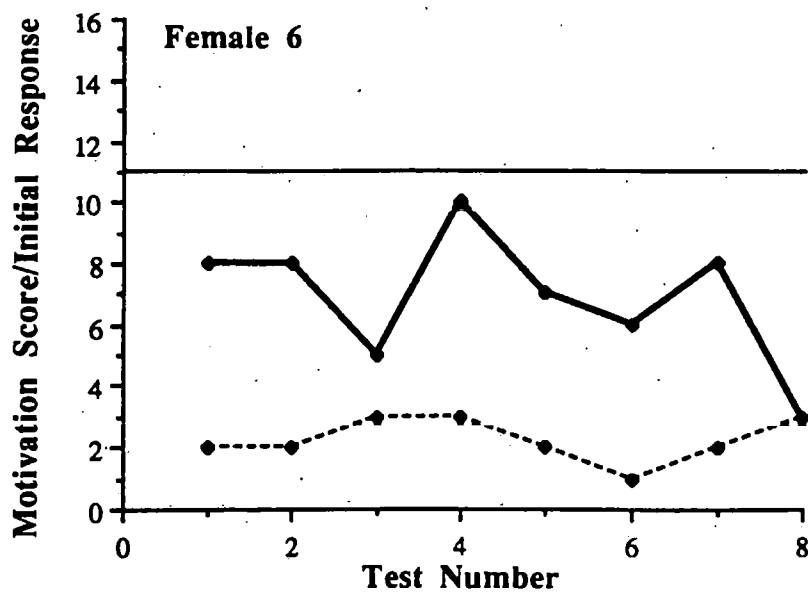
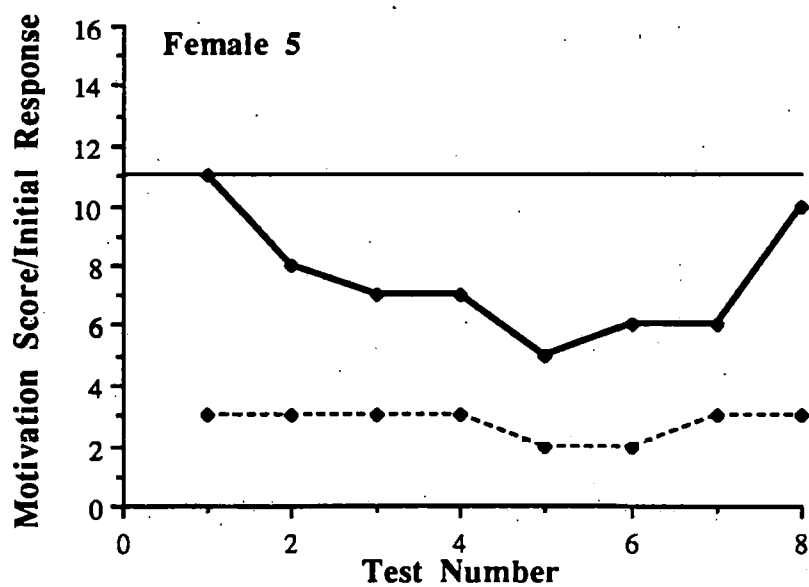
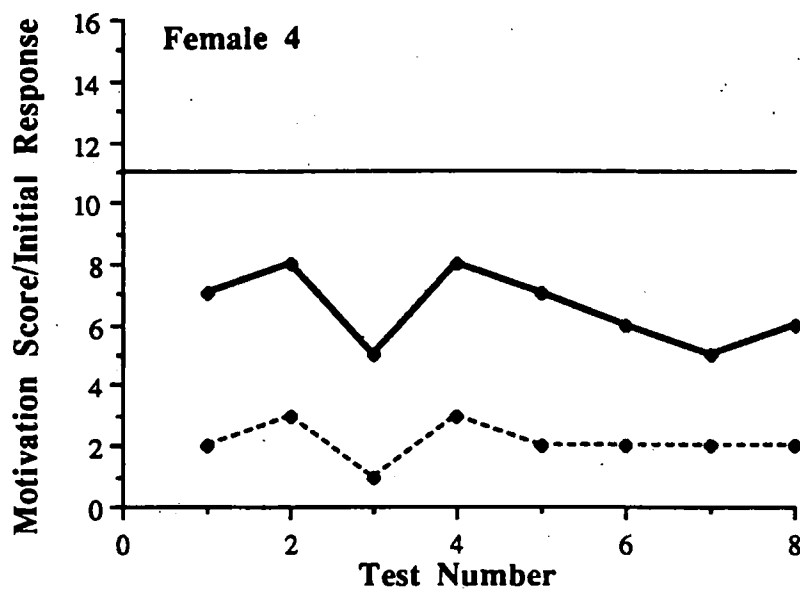
Looking at the profiles more closely, there seemed to be a threshold level of motivation, above which the female mated. In this study the threshold is represented by a motivation score of 11 (see Fig. 3.2). In addition to this apparent threshold there also seemed to be some kind of pattern to the females' responsiveness.

The successful tests were a reliable indication of the female's sexually highly motivated state at that time. It seems reasonable then to use this known state of responsiveness as a focal point. The motivation scores for each of the inseminated females in the tests before and after insemination were compared to see if the same pattern exists for all these females. Figure 3.3 shows this comparison around the focal point of insemination.

From this figure it appears that motivation fell to a low level in the test after that in which the female was inseminated. Interestingly however, it was also at a low level in



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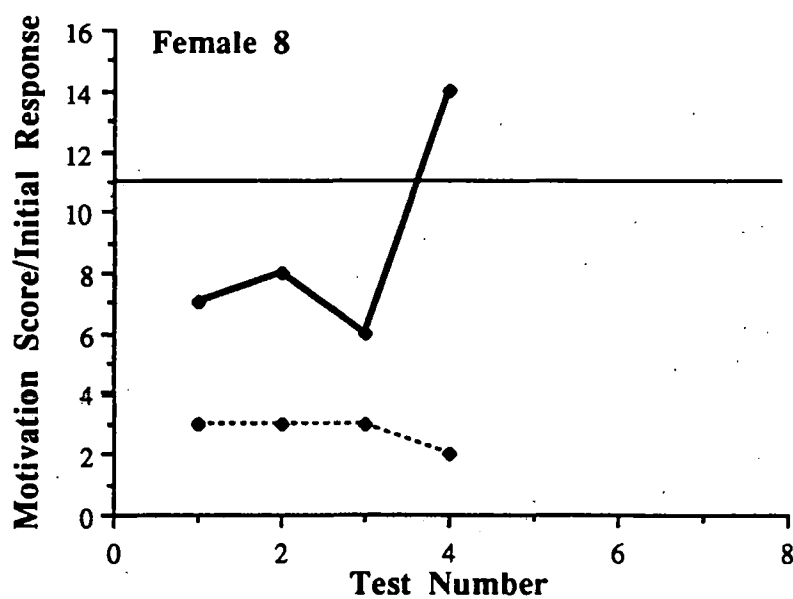
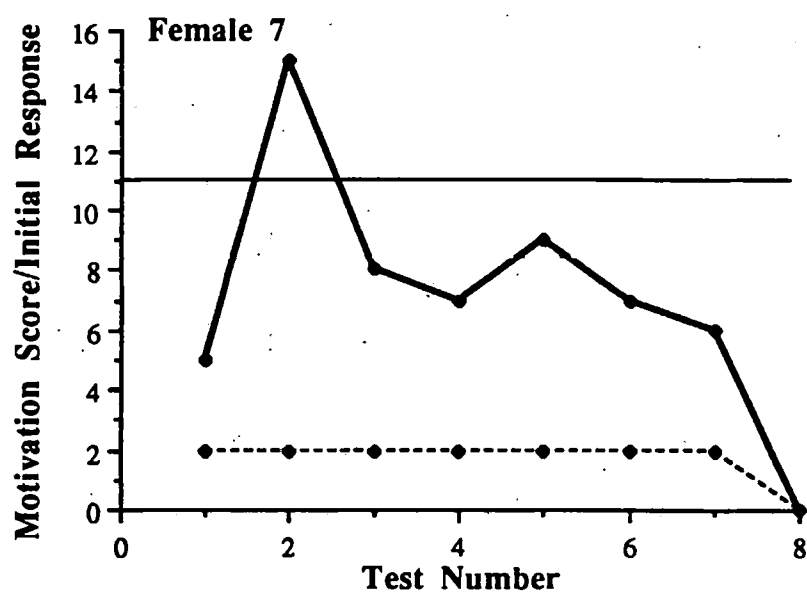


Fig. 3.2 Motivation profiles for all females over all tests. The dotted line shows response scores for the 'initial response' of the female. The solid line shows the response scores (total) for the 'courtship initiations' in each test. The line drawn at the motivation score of 11 is the threshold. (See text for details)

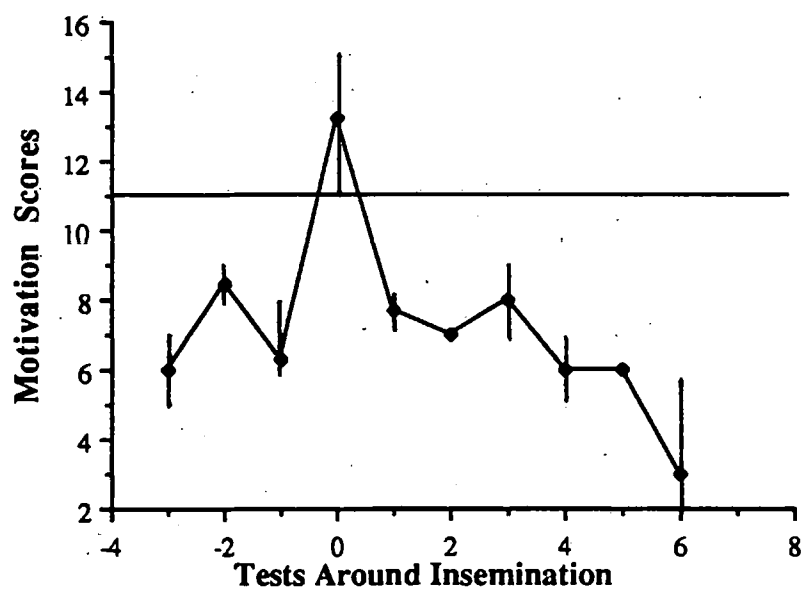


Fig. 3.3 Mean motivation scores for tests around the 'focal point' of insemination. (See text for details). The vertical bars indicate the range of values from which the mean was calculated.

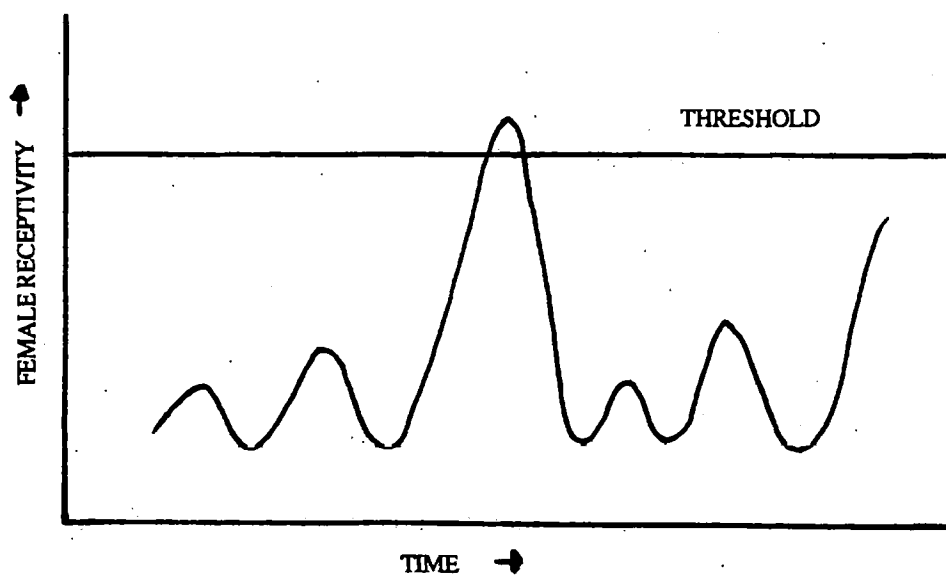


Fig. 3.4 Modified model of female responsiveness.

the test preceding the test where insemination occurred. This suggests that there was no graded increase in motivation through the tests leading up to the insemination test. There may, however, have been some more rapid increase in motivation occurring in the time between the tests, which was not detected here because of the time-scale of the tests.

Examination of this graph (Fig. 3.3) and the profiles for uninseminated females (see Fig. 3.2) indicates that female sexual motivation does vary over a long period of time (three weeks in this case). It appears to oscillate below some threshold level for a variable length of time. At some stage, under a set of as yet undefinable circumstances, the female becomes receptive and her motivation reaches or exceeds the threshold level; then she will respond to and mate with a male. Referring back to the simple model described in Fig. 3.1a, this model can be modified to reflect these findings, and this is shown in Fig. 3.4.

3.4.4 Female Motivation Over the Timescale of One Test

In general the responses of females towards males during a test could vary enormously. For example, females could be persistently negative, neutral or positive in response to the male's courtship initiations, or might be neutral in response to one or two courtship initiations and then negative in response to the rest. To illustrate this, Fig. 3.5 shows the responses of the four females that became inseminated, in the tests in which they became inseminated.

The profile for female 2 shows that her response to the first two courtship initiations was neutral, then her response to the third courtship initiation was positive with courtship continuing to the spermatophore transfer stage. Similarly female 8 went from being neutral to positive and a successful courtship was completed. Female 5, in contrast to 2 and 8, responded negatively to the first two courtship initiations and then suddenly responded positively to the next courtship initiation. In contrast again, female 7 responded positively to the first courtship initiation made by the male.

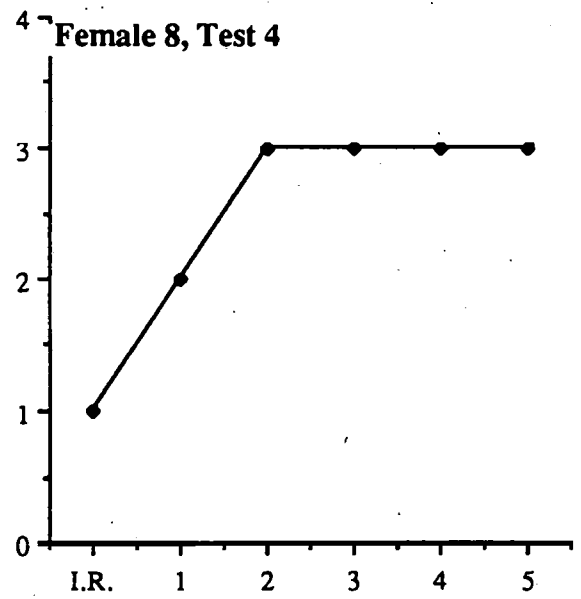
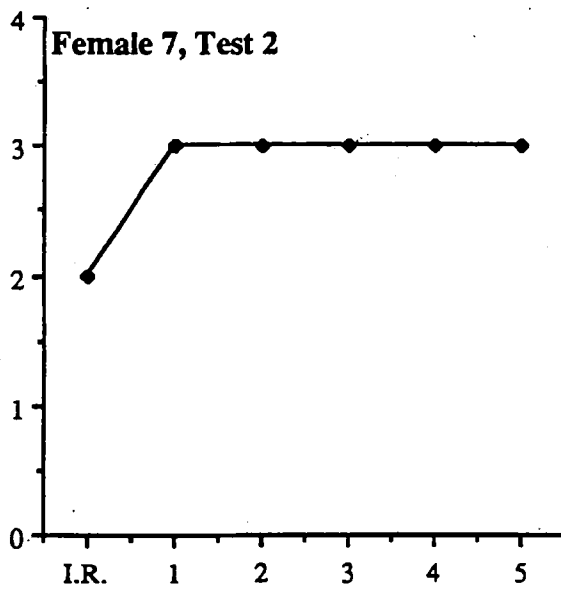
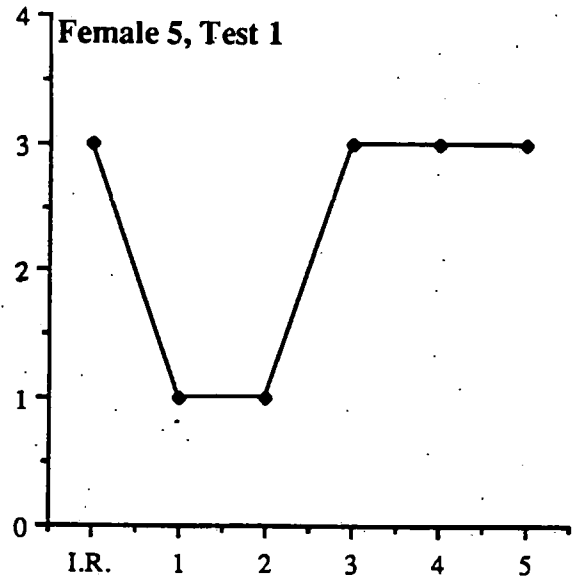
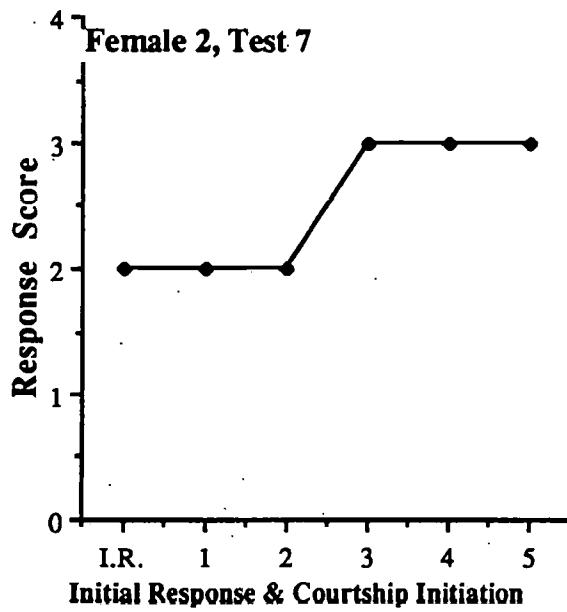


Fig. 3.5 Successful tests: the response scores over the tests where the four females shown became inseminated. (See text for details).

These profiles of motivation over the successful tests indicate that female motivation can be affected by the male's courtship. There seemed to be great variation amongst individual females in how quickly they responded to this courtship. This will of course depend upon their initial motivational state at the start of the test. Female 7, for example, was highly motivated at the beginning of the test, whereas other females (e.g. female 5) required more courtship before becoming responsive. The profile for female 5 shows that it is possible for a female's motivation to change from her being apparently completely unresponsive (negative), to being positive and mating with a male, over the time course of a single test (i.e. courtship encounter).

The effect of male courtship seems to last for only a short time, even within a single encounter. It was frequently the female that terminated a test where actual spermatophore transfer had taken place, by persistently walking away from the male, after picking up sperm from that male (also noted by Halliday 1974, Teyssedre & Halliday 1986). Male courtship does not appear to have a cumulative effect either, as there is no gradual increase in motivation over time (see Figs. 3.2 & 3.4). It seems, therefore, that male courtship may affect the female's motivation in the manner depicted in Fig. 3.1b. However, no spontaneous increase in receptivity was observed in this study.

3.4.5 The Initial Response to a Male

As well as the motivation profiles for each female over all the tests, Fig. 3.2 also shows the initial response scores for each test. These initial response scores, as discussed earlier, might be expected to give an indication of the female's motivational state. It was thought that the subsequent courtship initiation responses might reflect the initial response scores.

Fig 3.6 combines the data in Fig 3.5 for all inseminated females. It shows how the initial response of a female was very variable (c.f. Halliday & Teyssedre 1986, see

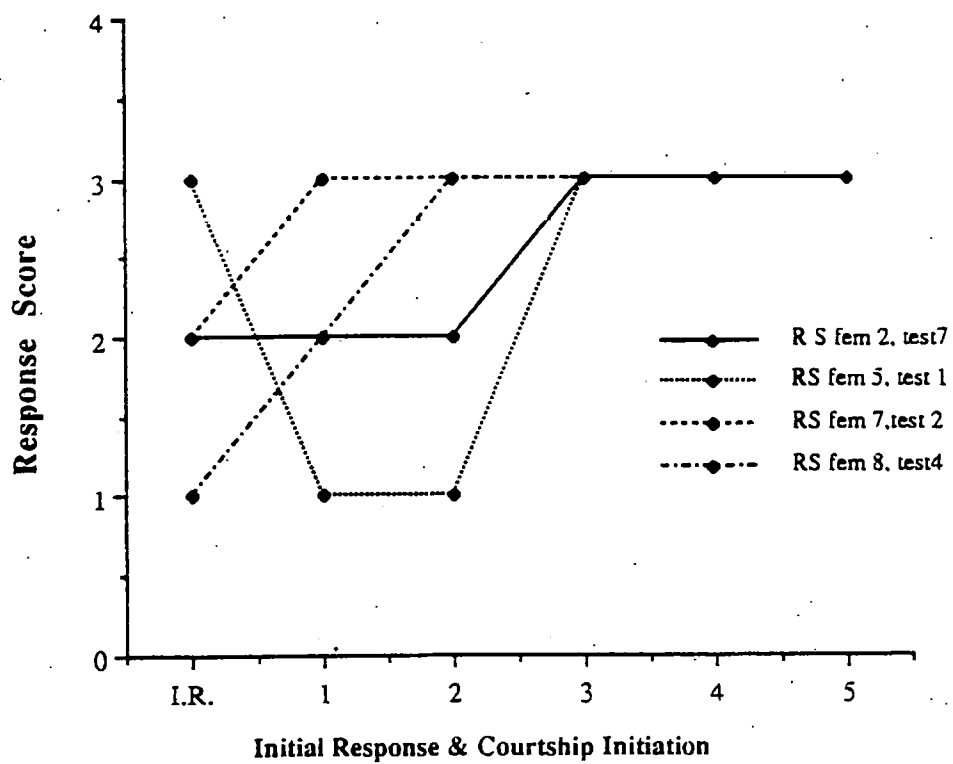


Fig. 3.6 Combined response scores for successful tests to show variation in initial response.

Section 3.5). Fig 3.6 also shows that initial response and courtship initiation response scores seem to bear no relationship to one another in successful courtships.

This relationship can be considered for all courtships (successful and unsuccessful). Although some female's initial response scores seem similar to the courtship initiation scores recorded in the rest of the test (see Fig. 3.2), others seem always to make a similar initial response regardless of their subsequent courtship initiation responses. For example, the initial response scores recorded for females 1 and 4 seem to reflect their courtship initiation responses, but female 7 generally made the same initial response whatever her later responses to the male's courtship were. No significant differences between the mean motivation scores for tests with initial response scores of 1 (mean=1.31;n=13), 2 (mean=2.45;n=29) and 3 (mean=2.65;n=17) were shown (Students t-test). Thus it appears that the initial response score did not provide a reliable indication of the female's motivational state.

3.5 Conclusions and Discussion

This work has shown that, at least over part of the breeding season, the sexual motivation of female newts may vary in the long and short term. Over a long timescale, motivation seems to oscillate below some threshold level, only rising above it under certain circumstances, when the female will mate. It then falls below the threshold level once more.

Over the short period of time that one test occupies, females can be initially unresponsive to a male, then become positive in response to courtship and pick up sperm and then become unresponsive once more. The effect of male courtship appears not to be long-lasting. There is no cumulative effect of courtship, at least as far as this study could detect.

The initial response that a female makes to the presence of a male appears not to be a reliable indication of her motivational state, as it does not consistently reflect her later

responses to male courtship. This suggests that Teyssedre & Halliday (1986) may have missed some potentially interesting information in their study. They recorded female responses to males and concluded from their observations that females were generally unresponsive to males at the beginning of a courtship encounter. This study found that females were not always unresponsive to males at the beginning of a courtship encounter. This was taken into consideration when planning the work that came out of this preliminary study.

A number of problems were encountered in this study that had an effect on the questions being investigated. In later tests it was noted that males were sometimes reluctant to court the females (see section 3.3.3). This may indicate that the sexual motivation of males could confound the issue of female motivation (this issue is taken up in more detail in Chapter 5).

Reluctant males were 'encouraged' with strait-jacketed females (section 3.3.3). Although this seemed a reasonable solution as males would on occasion court better after being encouraged, the effect that this novel stimulus might have on the male is not known. It may in turn have an effect on the test female as the male's courtship could have altered in some way after stimulation with the strait-jacketed female.

Another problem was that nothing was known about the previous history of the females. It was not known how long they had been in the pond, how many males they had previously mated with (if any) or whether they had been egg-laying. These are all factors that could affect their sexual motivation but which could not be controlled for in this study.

The way that female responses were categorised (negative, neutral or positive) meant that only a rough measure of motivation and of changes in motivation could be obtained. In particular, this meant that courtships could really only be classed as successful (sperm picked up), or unsuccessful (no sperm picked up). There is obviously

much more of a graded sequence of levels of responsiveness during a courtship, but these subtleties have not been highlighted or quantified in this study.

This work, completed in the 1988 season, was greatly expanded upon and improved in the following year, including resolving many of the problems outlined above. This work forms the the subject of the following chapters.

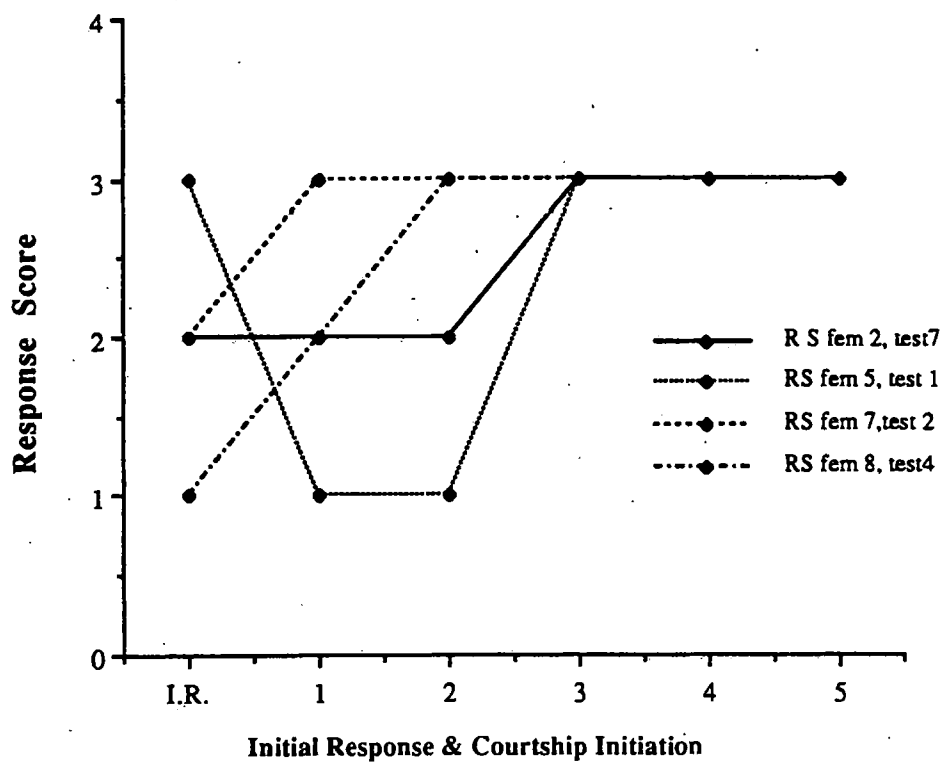


Fig. 3.6 Combined response scores for successful tests to show variation in initial response.

Section 3.5). Fig 3.6 also shows that initial response and courtship initiation response scores seem to bear no relationship to one another in successful courtships.

This relationship can be considered for all courtships (successful and unsuccessful). Although some female's initial response scores seem similar to the courtship initiation scores recorded in the rest of the test (see Fig. 3.2), others seem always to make a similar initial response regardless of their subsequent courtship initiation responses. For example, the initial response scores recorded for females 1 and 4 seem to reflect their courtship initiation responses, but female 7 generally made the same initial response whatever her later responses to the male's courtship were. No significant differences between the mean motivation scores for tests with initial response scores of 1 (mean=1.31;n=13), 2 (mean=2.45;n=29) and 3 (mean=2.65;n=17) were shown (Students t-test). Thus it appears that the initial response score did not provide a reliable indication of the female's motivational state.

3.5 Conclusions and Discussion

This work has shown that, at least over part of the breeding season, the sexual motivation of female newts may vary in the long and short term. Over a long timescale, motivation seems to oscillate below some threshold level, only rising above it under certain circumstances, when the female will mate. It then falls below the threshold level once more.

Over the short period of time that one test occupies, females can be initially unresponsive to a male, then become positive in response to courtship and pick up sperm and then become unresponsive once more. The effect of male courtship appears not to be long-lasting. There is no cumulative effect of courtship, at least as far as this study could detect.

The initial response that a female makes to the presence of a male appears not to be a reliable indication of her motivational state, as it does not consistently reflect her later

responses to male courtship. This suggests that Teyssedre & Halliday (1986) may have missed some potentially interesting information in their study. They recorded female responses to males and concluded from their observations that females were generally unresponsive to males at the beginning of a courtship encounter. This study found that females were not always unresponsive to males at the beginning of a courtship encounter. This was taken into consideration when planning the work that came out of this preliminary study.

A number of problems were encountered in this study that had an effect on the questions being investigated. In later tests it was noted that males were sometimes reluctant to court the females (see section 3.3.3). This may indicate that the sexual motivation of males could confound the issue of female motivation (this issue is taken up in more detail in Chapter 5).

Reluctant males were 'encouraged' with strait-jacketed females (section 3.3.3). Although this seemed a reasonable solution as males would on occasion court better after being encouraged, the effect that this novel stimulus might have on the male is not known. It may in turn have an effect on the test female as the male's courtship could have altered in some way after stimulation with the strait-jacketed female.

Another problem was that nothing was known about the previous history of the females. It was not known how long they had been in the pond, how many males they had previously mated with (if any) or whether they had been egg-laying. These are all factors that could affect their sexual motivation but which could not be controlled for in this study.

The way that female responses were categorised (negative, neutral or positive) meant that only a rough measure of motivation and of changes in motivation could be obtained. In particular, this meant that courtships could really only be classed as successful (sperm picked up), or unsuccessful (no sperm picked up). There is obviously

much more of a graded sequence of levels of responsiveness during a courtship, but these subtleties have not been highlighted or quantified in this study.

This work, completed in the 1988 season, was greatly expanded upon and improved in the following year, including resolving many of the problems outlined above. This work forms the the subject of the following chapters.

CHAPTER 4

Variations in Female Sexual Motivation - a functional viewpoint

4.1 Introduction

4.1.1 General

The work in this and the following chapter builds upon that just described (Chapter 3). The present chapter deals with variation in female sexual responsiveness and the adaptive significance of this variation. This provides a broad ecological background for the next chapter, which focusses on the causal aspects of variation in female responsiveness. First, a number of areas need to be introduced, to provide a context for the discussion of the study described here.

4.1.2 Overview of female seasonal cycle

A general discussion of the activities of newts over a year was given in Chapter 1 (Section 1.1). By way of a brief summary an outline will be reiterated here, from the female point of view. The newts migrate to the pond at the beginning of spring; they court and mate in the water; the females pick up sperm from males' spermatophores and it is stored in their spermathecae (Boisseau & Joly 1975; Halliday & Verrell 1984). They spend a large amount of time laying their eggs, then in summer, they emigrate from the pond and take up their terrestrial existence once more.

As already mentioned in Section 1.1.3, a very important aspect of the newt's reproductive cycle is that it appears to be dissociated (Crews 1987). In an associated breeding cycle, gonadal activity (i.e. the maturation and shedding of gametes {Crews 1987}) increases greatly just prior to the occurrence of mating; with a dissociated cycle gonadal activity is at a minimum during the mating period but increases greatly after mating. This dissociated pattern may also be referred to as post-nuptial gametogenesis (Verrell et al 1986).

This kind of breeding pattern occurs in species such as newts (see Verrell et al 1986) which have a regular but narrow opportunity for breeding (Crews 1987) and

this has an important feedback to the mating strategy employed by each sex (see Section 1.1.3). Females arrive at the pond with their full complement of eggs for the season (although they may not all be fully yolked at this stage, Verrell et al 1986) and, after insemination, go on to lay them over the course of the season. This part of the project looked in more detail at these activities and their relationship with one another.

4.1.3 Multiple mating and the ecological context of mating

It is easy to see why it is advantageous for males to mate with more than one female since, relative to females, they tend to produce many more gametes. There is increasing evidence that males benefit from multiple mating by increasing their reproductive output (Birkhead & Hunter 1990a). However, it has become evident that females of many species mate more than once and there are many reasons (see Knowlton & Greenwell 1984; Parker 1984; Halliday & Arnold 1987 and references below) why these females might mate multiply. For example, it may be adaptive for them to mate with a better male than their initial choice, or to acquire sperm from more than one male so that their progeny are genetically diverse. However, perhaps they simply require more sperm than is obtained from one mating to fertilize their whole clutch of eggs, if, as is the case for newts, they have them in large numbers. The important relationship between multiple mating and sperm competition is discussed later (Section 4.5).

The phenomenon of multiple mating has been described for many species including insects (Taylor 1967; Pease 1968; Byers 1978; Pyle & Gromko 1978; Sakaluk & Cade 1980; Otronen 1989), lobsters (Nelson & Hedgecock 1977), fish (Chesser et al 1980; Travis et al 1990), birds (see Birkhead & Moller 1992, for a review) and discussed from a more general perspective (Pyle & Gromko 1981; Halliday & Arnold 1987).

Amongst urodeles, genetical and biochemical studies have been conducted which revealed multiple paternity of egg clutches from a single female (in *Triturus alpestris*, Rafinski 1981; in *Desmognathus ochrophaeus*, Tilley & Hausman

1976, Houck et al 1985, Labanick 1986). A laboratory experiment by Verrell (1984b) indicates that female smooth newts can mate more than once, but it appears to have been a rare event (2/25 cases) in that study (discussed in Halliday & Verrell 1984).

It is important to determine the ecological context in which mating takes place for a number of reasons. The variations in female sexual responsiveness can only be understood if any conflicting activities the female may have to engage in have been identified. Similarly, certain environmental conditions (e.g. density of individuals in the pond, limited food supply etc.) may impose constraints upon female behaviour. Knowledge of aspects of the ecological and social background to mating are crucial to the understanding of mate choice and to the design of mate choice experiments (e.g. see Sullivan 1990).

4.2 Aims

The primary aim was to monitor the sexual motivation of females over the whole breeding season, in a longitudinal study, and to identify any factors that may affect it. Previous studies that have included aspects of female behaviour have found females to be generally unresponsive (Halliday 1974; Verrell 1983; this study, Chapter 3). The animals for those studies were collected from ponds after the beginning of the breeding season. Their sexual history was, therefore, unknown and could have made a difference to females' receptivity. The present study aimed to eliminate this problem.

To this end, the individual sexual histories of eight females were studied, from their collection on migration to the pond, through mating and egg-laying, until they became terrestrial once more some twelve weeks later. Eight females were used, as the preliminary study indicated that this was the most practicable number that could be tested repeatedly as the study required. The specific aims were as follows:

- 1) to document the occurrence of inseminations over the breeding season and to observe whether a general pattern emerged over time;
- 2) to chart each female's egg-laying activity over the season and to monitor any effect it may have had on female sexual responsiveness;

3) to monitor the effect of temperature on the females' activity.

4.3 Materials and Methods

Chapter 2 described the capture and maintenance of the animals in detail. The animals used were collected as they migrated to their breeding pond in early-mid March 1989. Eight females were housed individually in tanks and the males were kept in groups in tanks. All were kept under semi-natural conditions, either in the outdoor shed, or in the large buffered tubs.

The experimental procedure explained in Section 2.3 (see also Section 3.2.2) was followed. The females were presented with a male every other day (early a.m. or late p.m.) from 21st March until late May. The outcome (i.e. whether the female became inseminated or not) was recorded for each test. (Further detailed information was recorded but this is the subject of the following chapter.) Artificial weed was provided upon which the females could lay their eggs. The eggs were collected and counted every other day and the temperature was also recorded. After two of the females had ceased egg-laying and were becoming terrestrial once more, the tests were terminated and these females were returned to the pond edge. The remaining females were kept until they also ceased egg-laying and became terrestrial; they were then also returned to the pond.

4.4 Results

4.4.1 Pattern of inseminations

Previous work on female receptivity showed that females were generally unresponsive when the male attempted courtship. This could be explained by a number of factors; females may be very choosy, their sexual motivation may be generally low or very variable, or the experimental conditions may simply not have been conducive to mating.

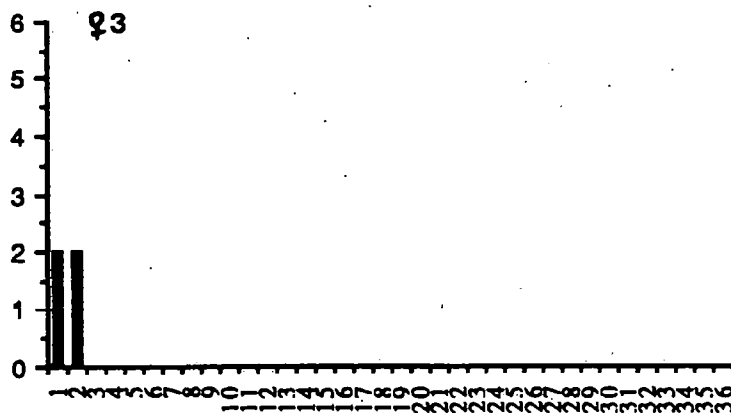
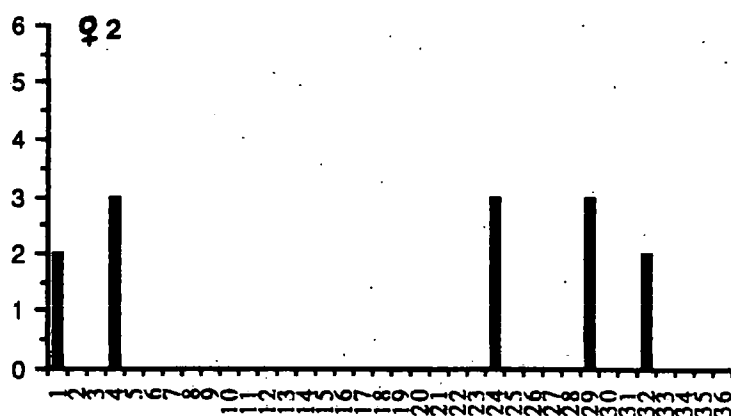
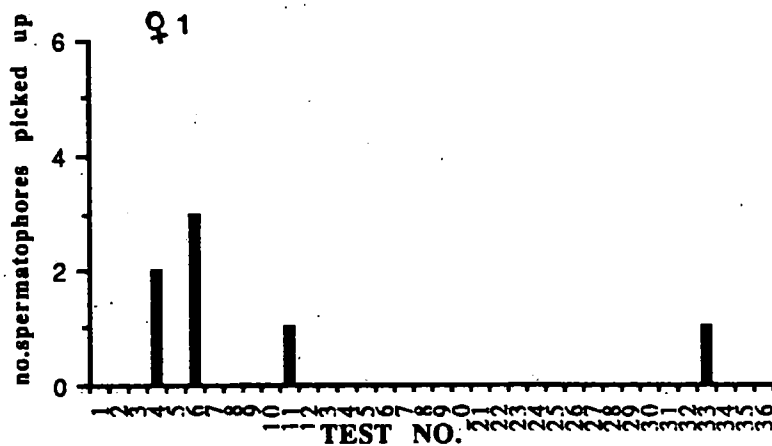
In this study the females were collected on migration which meant they had not yet had an opportunity to mate in that season. Thus the first male with which they

were presented (in the first test) was the first male with which they had the opportunity to mate.

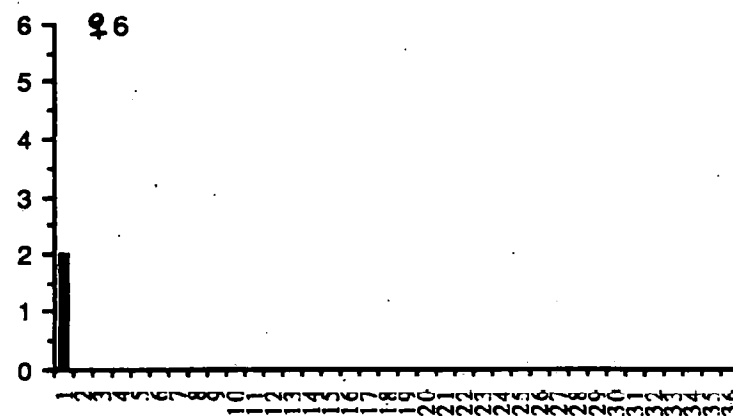
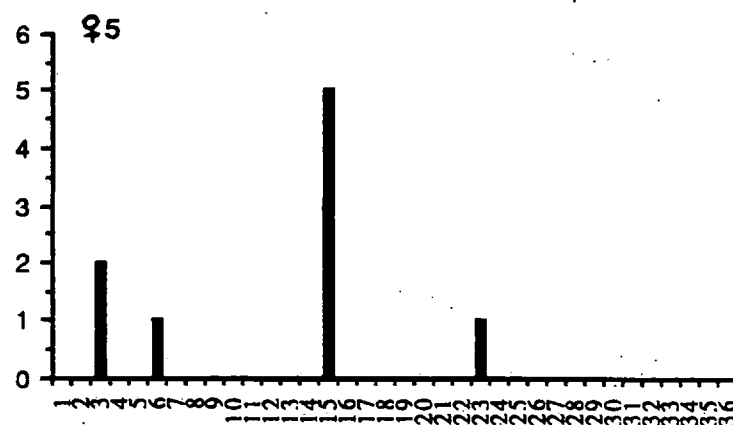
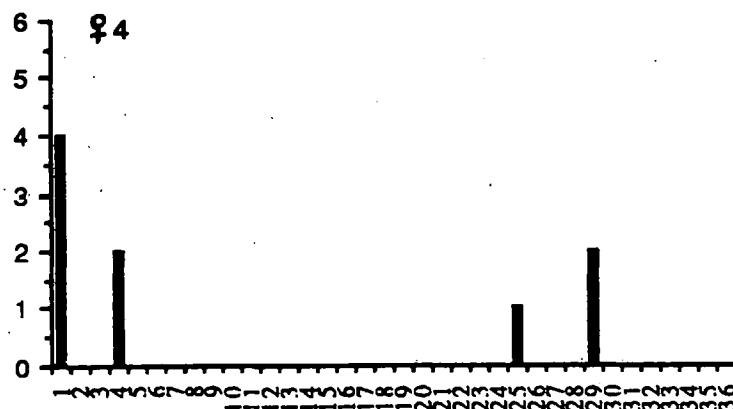
Contrary to other findings, the females in this study were not generally unresponsive to males. Considering the first test, seven out of the eight females elicited the deposition of at least one spermatophore from the male with which they had been presented. Six out of eight females picked up at least one of the spermatophores deposited by the male in this first test. (Recall that in the earlier work done for this project {see Chapter 3} over all the 64 tests observed only 4 inseminations occurred.) In addition, some females were very responsive towards the males. They frequently approached the males to elicit courtship before the males had approached them first, which is more usually the case. I have termed this 'proceptive' behaviour (Beach 1976) and it is discussed more fully in the next chapter.

Over the rest of the season, after this first test, further inseminations occurred. During the whole season, female 3 was inseminated by two different males, i.e. on two different occasions; female 8 was inseminated on three occasions; females 1, 4 and 5 on four occasions and females 2 and 7 on five occasions. Female 6 alone was inseminated only once. She was smaller than the other females, which may have made her less attractive to the males (Verrell 1986b), or she may not have been sexually mature. She also did not lay any eggs although she appeared fairly plump at the beginning of the season. Baker (1990a) has also found that very small females tend not to lay eggs, despite having been inseminated.

The pattern of inseminations was examined for all the females. Fig 4.1 shows the distribution over the season of each female's inseminations. It can be seen that many of the females mated more than once in the initial stages of the season (i.e. from the beginning of the time in which the females were tested). When the data for all the females are combined a clearer pattern begins to emerge. Fig 4.2 shows the number of females inseminated on particular test days. There appears to have been a period of intensive mating over the first six tests, after which inseminations occurred less often, and fewer females became inseminated in any one test.



continued



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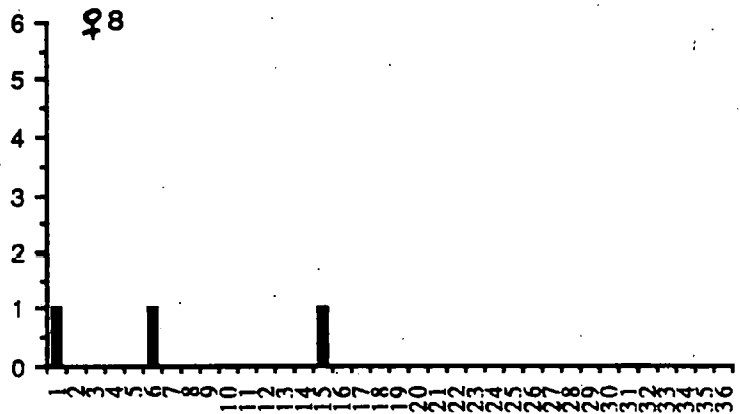
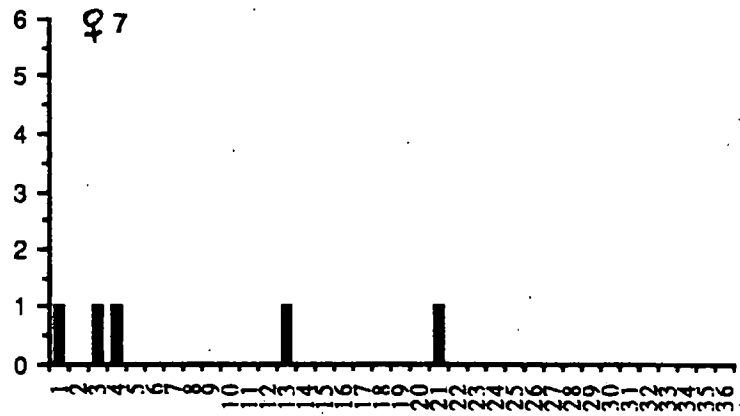


Fig. 4.1 Distribution of inseminations over the season for each female. The vertical bar indicates the test in which insemination took place. The height of the bar indicates the number of spermatophores picked up by the female in that test. (See text for details).

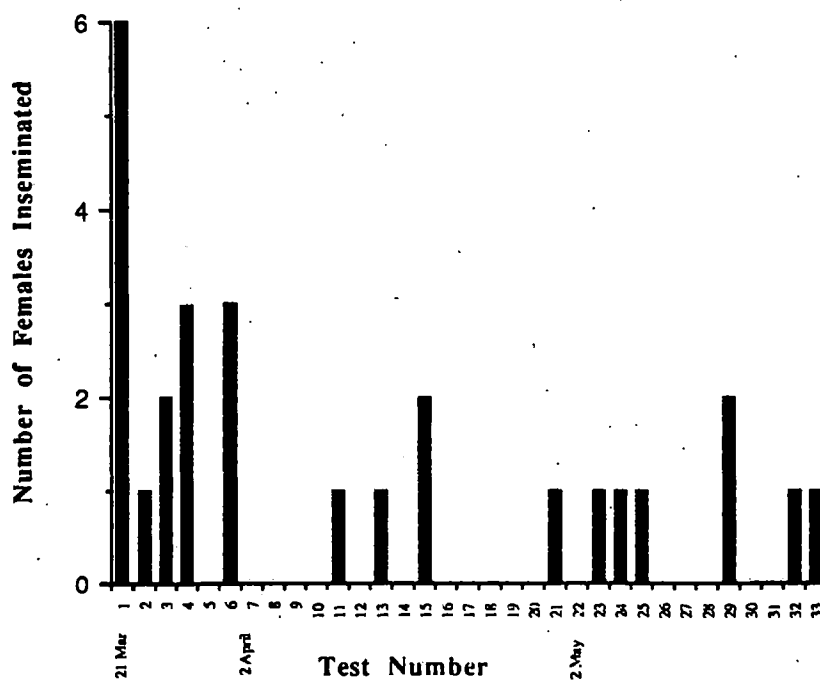


Fig. 4.2 Combined data for female inseminations (N=28) showing the number of females inseminated on particular test days. Dates are shown to indicate the passing months.

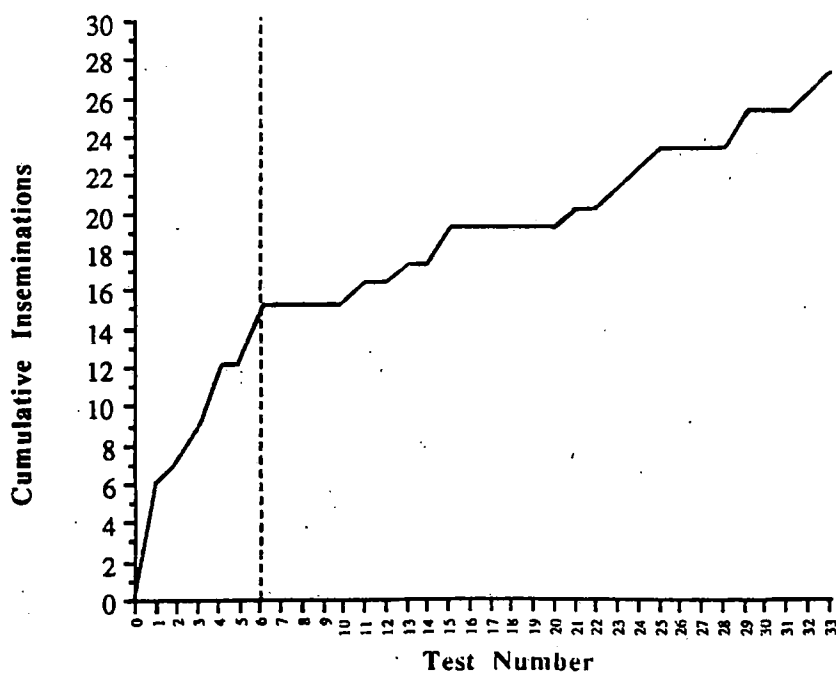


Fig. 4.3 Cumulative analysis of female inseminations over the season, showing over half the total inseminations occur in the first six tests.

Cumulative analysis (see Fig 4.3) shows that there was a much lower rate of insemination after the sixth test. Of the 28 inseminations that occurred over the whole season, 15 occurred in the first six tests with the remaining 13 spread over 27 further tests.

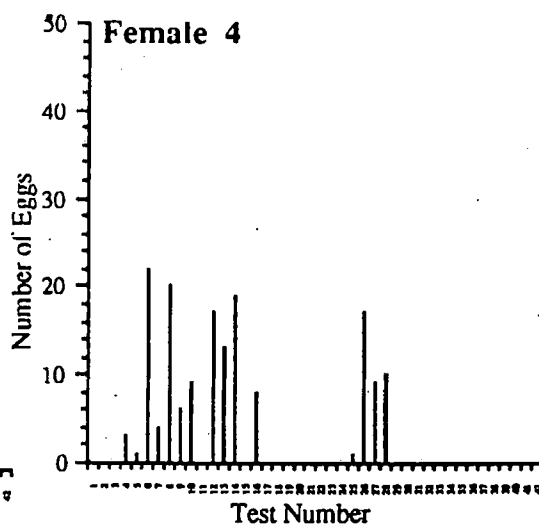
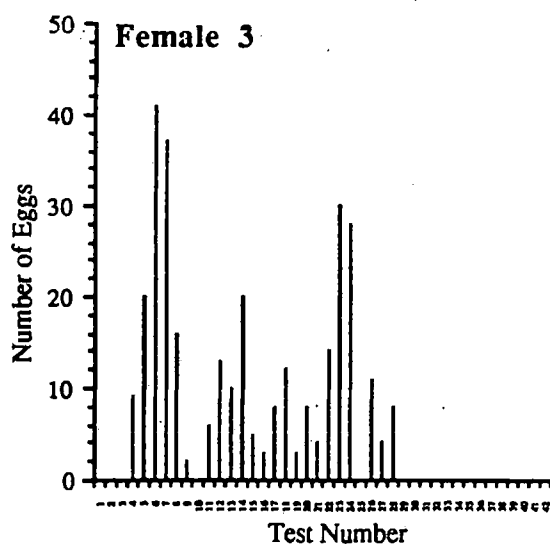
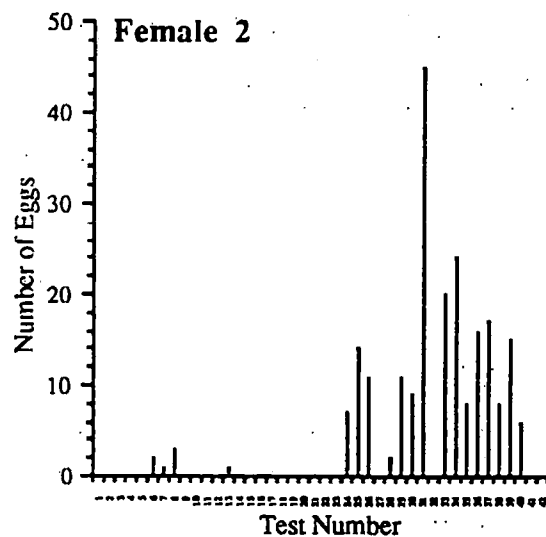
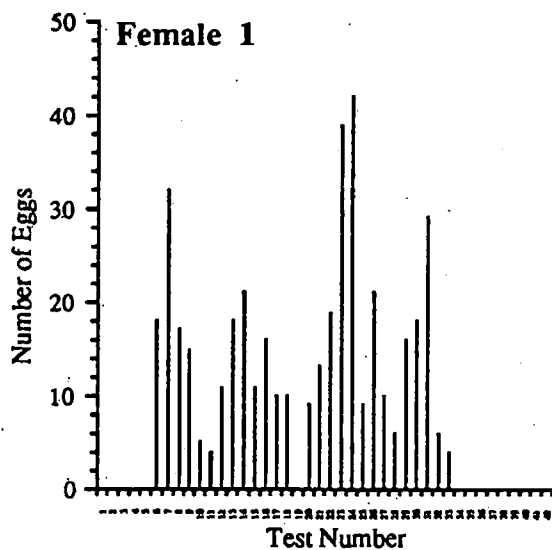
Thus these results show that females are not generally unresponsive; they appear in fact to be sexually very highly motivated at the beginning of the season. In this experiment the beginning of the season was represented by the first six tests, which is around twelve days from the females first being presented with a male. After this they became less receptive to male courtship and mated less often.

These results also show that females do mate multiply and, especially when the whole season is taken into account, that it is not a rare event. All except female 6 had mated with at least two different males (i.e. on two different occasions) by the end of the sixth test (see fig 4.1). Around this time the females began laying eggs in large numbers, as the next section reveals; thus they had multiply mated before egg-laying commenced. This means that sperm from more than one male will be present in the spermatheca and sperm competition could, potentially, take place from this stage onwards, over the season, with sperm from other males added later.

4.4.2 Insemination and egg-laying

The second aim of this study was to chart each female's egg-laying activity over the season and to monitor the effect of egg-laying on female receptivity. The number of eggs each female laid was counted every other day. The pattern of each female's egg-laying activity is shown in Fig 4.4. The patterns varied between individuals (also found by Baker 1990a) but some general features could be pinpointed here. After the initial insemination all the females, except number 6, began to lay eggs between six and ten days later. This egg-laying activity continued over the season but with breaks, of varying duration, for some females (see Fig 4.4).

It was mentioned earlier that the ecological context of mating is important. Here, it appears that egg-laying may be a conflicting activity for the females. Fig 4.5



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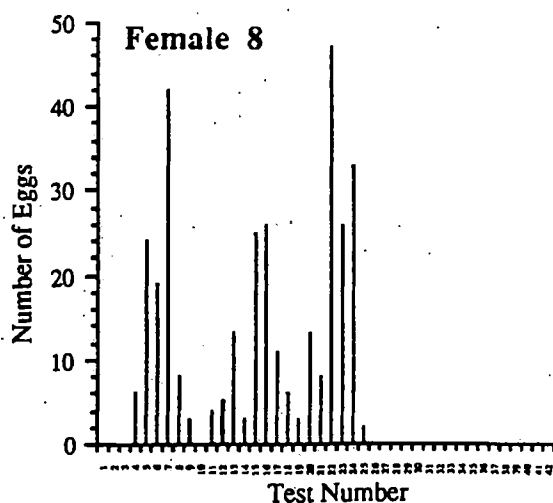
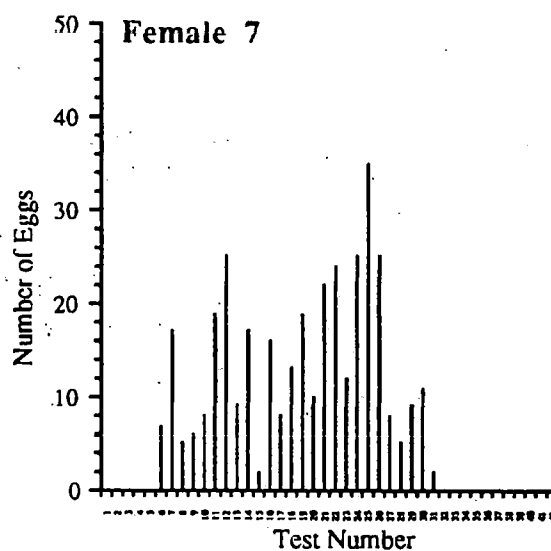
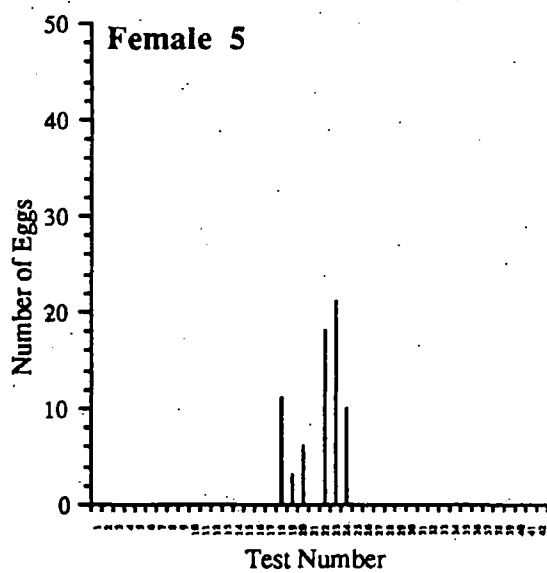


Fig. 4.4 Patterns of egg laying shown by individual females. The graphs show the number of eggs counted on each test day. NB Female 6 did not lay any eggs at any time over the season.

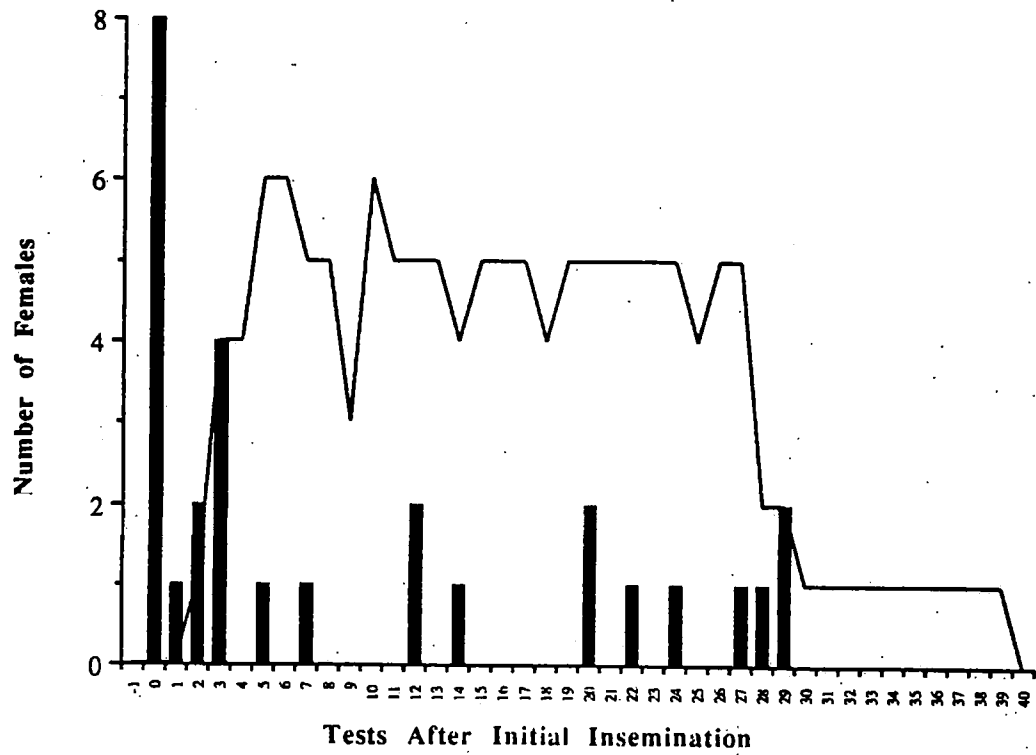


Fig. 4.5 Interactions between inseminations and egg laying over the season. The vertical bars indicate the number of females inseminated. Test 0 is the first test in which each female became inseminated. (See text for further details). The solid line indicates the number of females egg-laying on that test day.

shows the overall pattern of inseminations and egg-laying for all the females. The information is centred around the first test in which each female became inseminated. Thus, this was test 4 for female 1, test 1 for female 2, test 1 for female 3 etc. It was thought that there may be a causal link between insemination and egg-laying, so the data were focussed around the first insemination test to standardise the information. This potential causal link is considered more closely in the next chapter.

The general pattern seems to be that the short intensive mating period occurs (as already shown in Section 4.4.1) and almost immediately the females begin to lay their eggs. This continues over the season with further inseminations occurring from time to time (as shown in Fig 4.5).

The total number of eggs laid over the season by each female is also of interest, since this may be related to the number of matings engaged in. Females may mate only as often as is necessary to obtain enough sperm to fertilize their clutch. Thus a relationship might be expected between the total number of spermatophores picked up and the total number of eggs laid over the season.

Referring back to Fig 4.1 it can be seen that there was considerable variation in the number of spermatophores a female picked up on a particular occasion. (The mate choice aspects of this will be discussed in Chapter 6.) Snout-vent length (SVL) is used as a convenient measure of female size. (It is closely correlated {Spearman} with female overall length {snout tip to tail tip}, $r = 0.952$; $p < 0.01$.)

Firstly, the relationship between female size and the total number of eggs laid over the season was examined. No significant correlation was found for this group of females (Fig 4.6) although this relationship has been found previously (Baker 1990a). In addition, no relationship was found between the total number of spermatophores picked up over the season and the number of eggs laid (Fig. 4.7). The significance of these results is discussed later in Section 4.5.

4.3.3 The effect of temperature

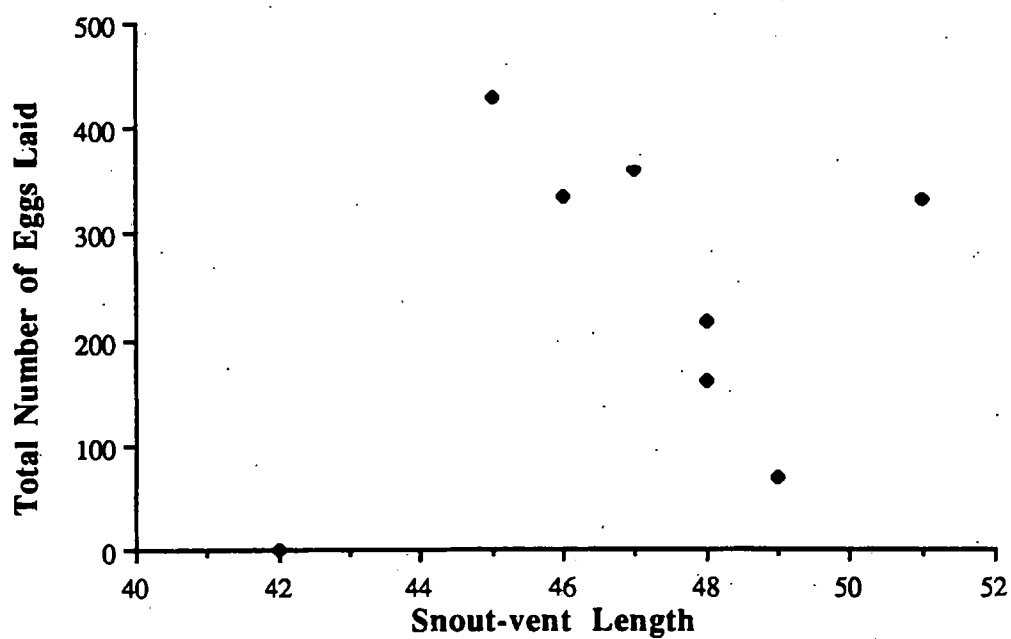


Fig. 4.6 Relationship between SVL and the total number of eggs laid over the season, for the eight females.

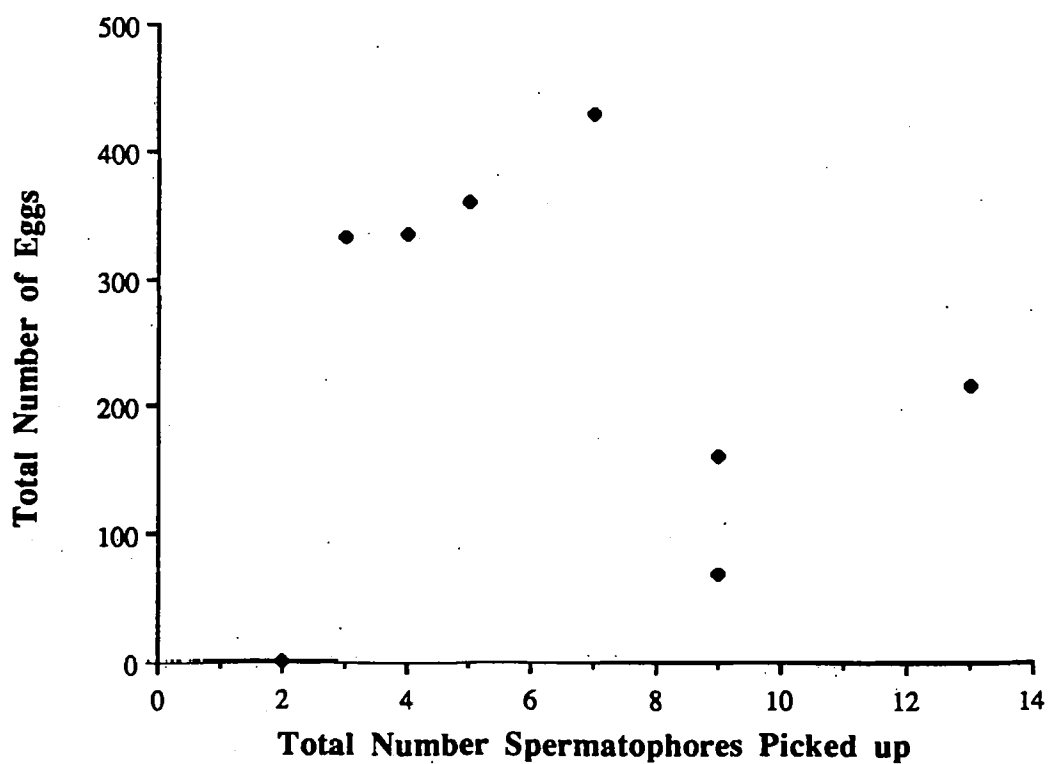


Fig. 4.7 Relationship between SVL and the total number of spermatophores picked up over the season, for the eight females.

The numbers of eggs were counted every other day and the temperature was also recorded. Thus, egg-laying rate could be calculated and was related to temperature. It could then be used as an indicator of activity levels. The rate of egg-laying over the two days prior to the test was also worked out from the number of eggs counted. A mean temperature for the time in which these eggs were laid was worked out. This was done by taking the mean of the temperature at the end of the previous test day and that at the beginning of the test day when the eggs were counted.

Fig 4.8 shows that, if temperature is high, females lay eggs at a faster rate than if the temperature is low. Fig 4.9 shows how egg-laying rate and temperature vary individually, and together, over the season.

Temperature evidently exerts a great influence on egg-laying rate. It is quite likely that low temperature reduces female activity generally and may well interfere with sexual responsiveness in this way. The consequence of this is discussed below.

4.5 Discussion

The major findings of this study are as follows.

i) Females mated repeatedly and with different males, which introduces the possibility of sperm competition in this species (Halliday & Verrell 1984; Verrell 1984a).

ii) Repeated mating occurred frequently at the very beginning of the season, egg-laying began six to ten days after the initial insemination and continued, with occasional further matings, over the season.

iii) Temperature had a marked effect upon the rate at which females could lay eggs. This may reflect a general constraint on female behaviour.

Since this study followed the histories of individual females under semi-natural conditions, the variations in female responsiveness can now be better understood in an ecological context. Obviously the study of the sexual dynamics of a natural population would provide the best idea of what is happening over a season in

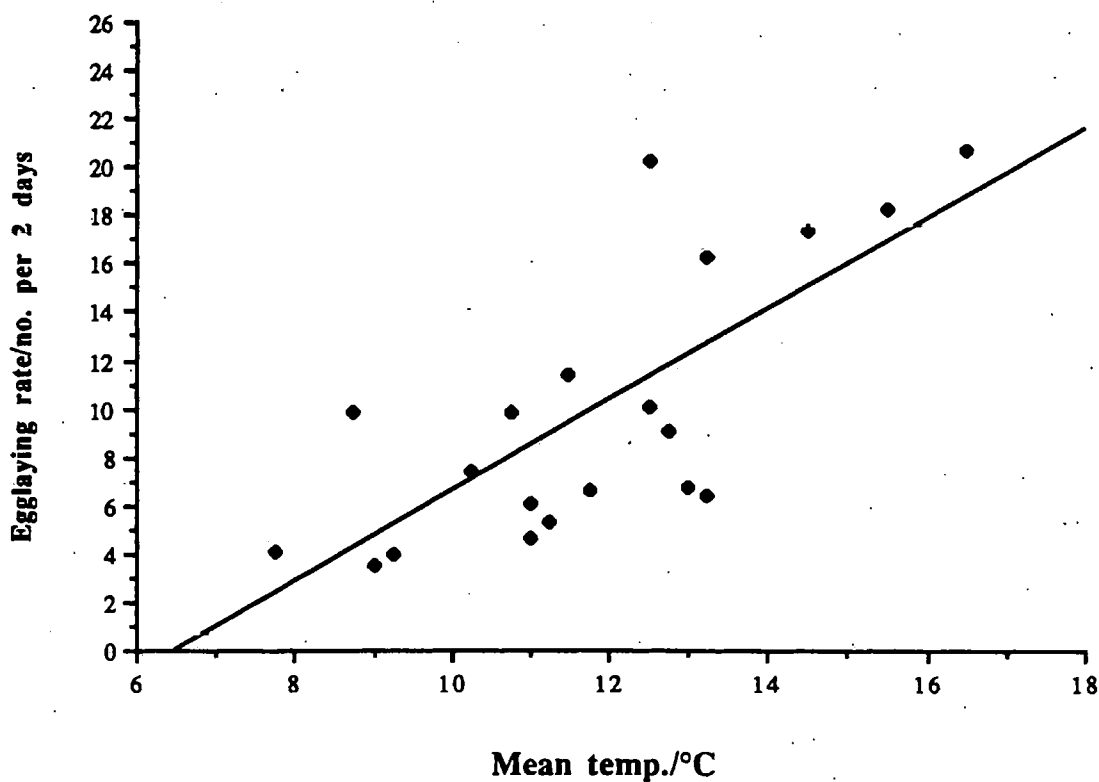


Fig 4.8 Correlation between mean egg-laying rate and mean temperature. $r = 0.74$; $p < 0.01$.

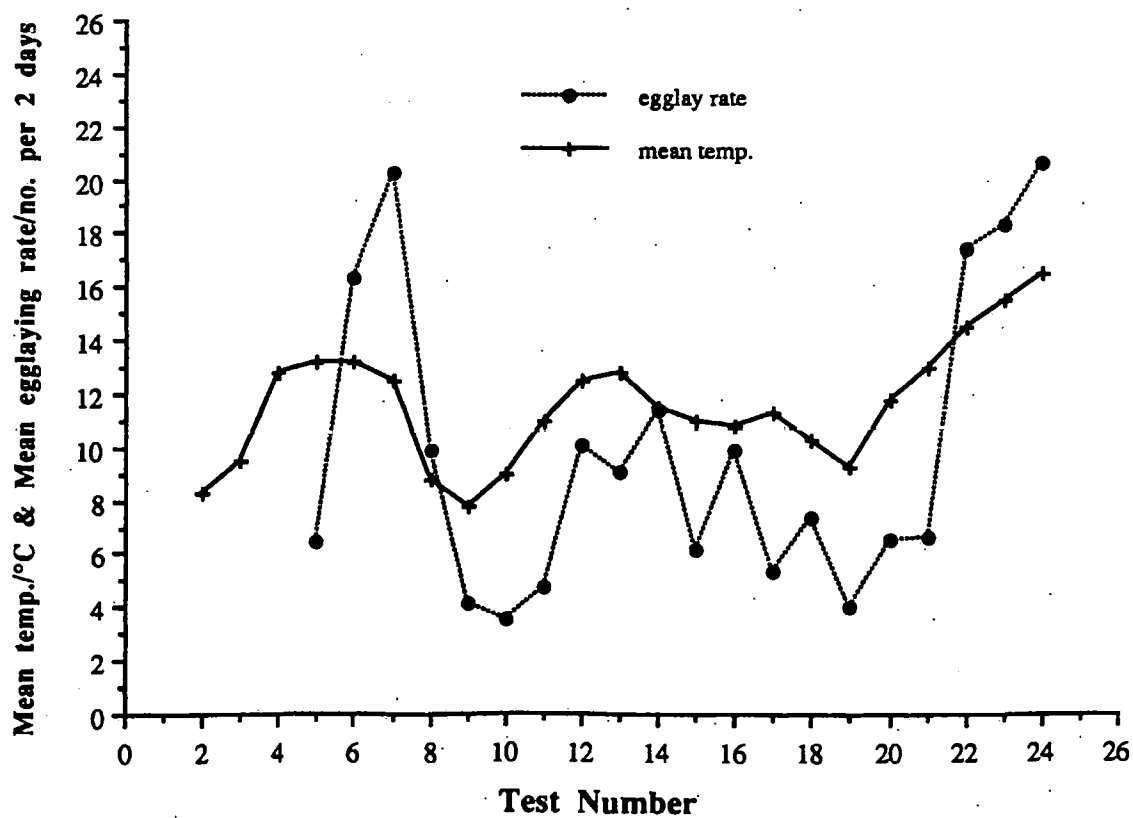


Fig 4.9 Variations in mean egg-laying rate and temperature over the season.

the wild, but this is extremely difficult. Verrell & McCabe (1988) carried out such study. They outline the difficulties in trying to observe shy animals, at night, in what is often less than clear water. By inference from observation of pairs of individuals in close proximity, they suggest that most sexual activity occurs at the beginning of the season. The present study bears this out and gives a more quantified idea of the amount of sexual activity occurring at this time. Intensive observations of a semi-natural 'population' would be an ideal next step in this line of investigation.

Females have been found to mate repeatedly with different males but the question of whether they are discriminating between different males remains. It is known that males develop their secondary sexual characteristics in the breeding season (Section 1.1.2) and these seem good candidates for female choice (Section 1.3.5). However, Griffiths & Mylotte (1988) showed, interestingly, that the males do not develop their crests maximally until well after the beginning of the breeding season.

The present study found that females mated most in the very early part of the breeding season, when the males would not have developed their secondary sexual characteristics to their greatest extent. In addition, the females did not appear to be very discriminating, at least at first (7/8 elicited sperm deposition from the first male with which they were presented). This interesting conundrum and the discussion of its solution forms a large part of Chapter 6.

The question remains of why mating occurs mainly in an intensive period at the beginning of the season and not, for example, regularly over the season. Bearing in mind the ecological background of the breeding season, the female's reproductive strategy should be considered.

Each female has a clutch of up to 600 eggs (Baker 1990a; present study, Fig 4.4) to lay over the season. Although there is some evidence to suggest that early-hatching amphibian eggs may have an advantage over late-hatching ones (e.g. Morin et al 1990), a simple time constraint may well be of overwhelming importance; the time needed to physically lay a large clutch of eggs may be quite considerable.

For *Triturus marmoratus pygmaeus* Diaz-Paniagua (1990) recorded a time of about five minutes to lay a single egg. This study recorded the number actually laid over the previous two days, from which a rate (eight eggs per day) could be worked out. For a clutch of about 300 eggs and laying at a rate of about eight eggs per day, a female would need around 40 days to lay the whole clutch. If the pond is likely to dry up in early summer, it is clearly adaptive for the females to get to the pond, become inseminated as soon as possible and begin egg-laying. If the spring is very cold, her rate of egg-laying would be slowed, which may add further to the problem of laying her clutch as quickly as possible. However, ponds may dry up more slowly in colder weather, which may counteract this to some extent.

A study of amphibians in Poland (reported in Frazer 1983) found that, in early April, female *Triturus vulgaris* laid eggs at a rate of one a day. In May, when the temperature rises to 15° - 22°C, females laid two eggs a day. Oviposition continued until late July or even August when the weather was cool. Clearly temperature is a major constraint. Even if the lower temperature means ephemeral ponds may be less likely to dry up, the female still has a time constraint: she must leave the water at some stage, in order to feed and replenish her gamete supply in the terrestrial phase.

Mating early in the season is evidently adaptive but why should females mate repeatedly? Ephemeral freshwater ponds, by their very nature, tend to be very variable (Dudley Williams 1987) and contain diverse micro-habitats (Baylis 1981). One of the reasons often suggested for multiple mating is that it ensures that diverse progeny result from a single clutch (eg. Halliday & Arnold 1987). In a very variable and unpredictable environment, it would be an advantage to have progeny that are genetically diverse. Recently, Madsen et al (1992) have made the interesting discovery that female adders (*Vipera berus*) that mate multiply have more viable offspring than those that mate less often.

This last is a good adaptive reason for why females remate. However, there are other possible explanations which are based more on physiological considerations. It has been thought that a single spermatophore contains enough sperm to fertilize the

whole of a female's clutch of eggs (Houck pers. comm. in Halliday & Verrell 1984). Pecio (1992) also believes this is the case; she reported that there are, on average, 'several hundred thousand' sperm in a single spermatophore. Recently Waights (pers. comm.) has developed a technique whereby the amount of DNA contained in a spermatophore can be calculated. This means the number of sperm contained therein can also be worked out (Waights, pers. comm.). She has found that there are between 3000 and 12000 sperm in a single spermatophore.

However, it is known for other newts (*Cynops spp.*) that between two and twenty sperm are required for the fertilization of a single egg (Iwao & Elinson 1990); i.e. they are polyspermic. If smooth newts are also polyspermic to this degree then there may not be enough sperm in a single spermatophore to fertilize a female's entire clutch. Also, Waights has found that the amount of sperm contained in a spermatophore may be very different, both when compared between different males, and between spermatophores from the same male (pers. comm.). These findings may explain why this study found no relationship between the total number of spermatophores picked up and the total number of eggs laid (Fig 4.7). Pecio (1992) found some evidence for such a relationship but she found varying numbers of infertile eggs. This area evidently needs further investigation.

Other aspects of sperm physiology may be important here. Sperm may die after some time in the spermatheca; the longevity of sperm is reviewed for urodeles by Halliday & Verrell (1984). All the sperm in a spermatophore may not be viable in the first place, or it may be that some essential nutrients supplied with the sperm may become depleted over time. This area of sperm viability etc. has not, unfortunately, received much attention as yet. The way in which females use the sperm they pick up and whether they utilise it efficiently also needs further study (Halliday & Verrell 1984).

Thus depletion of viable sperm supplies (or associated nutrients) is a possible explanation for remating in female newts. It may also suggest a causal mechanism by which the behaviour could be expressed. The idea of a simple negative feedback

system for control of remating has existed in the insect literature for some time (e.g. Taylor 1967; Raulston et al 1975). In Raulston's study, females of the tobacco budworm (*Heliothis virescens* F.) remated more if they had no sperm than if some were present. In a lepidopterid, Taylor found multiple mating was significantly less common in females that were well supplied with viable sperm. Female smooth newts are similar to insects in that they have a spermatheca, which enables sperm to be stored within the female's body (Boisseau & Joly 1975; Halliday & Verrell 1984). The suggestion that a simple system of stretch receptors in the spermatheca may control remating in insects (Taylor 1967), could point to a similar mechanism in newts; this possibility requires further investigation.

A final area that needs discussion with respect to multiple mating is sperm competition. According to Parker (1970), sperm competition is the competition between ejaculates from two or more different males over the fertilization of ova. It has already been made clear that the potential for it to occur does exist in *T. vulgaris* (i.e. females can store sperm and they mate repeatedly in a single breeding season).

Halliday & Verrell (1984) review the sperm competition situation for urodeles. They distinguish two possible kinds of sperm competition, internal within the female and external to the female. In the salamander *Ambystoma tigrinum* male-male competition can occur (Arnold 1976). It takes the form of 'spermatophore stacking' (Arnold 1976) where one male places his spermatophore directly on top of that from another male. Halliday & Verrell (1984) consider that this can be regarded as a form of sperm competition, external to the female, because 'the first male's sperm is rendered quite inaccessible to the female'. I would suggest that this is a form of male-male competition, not sperm competition: if the sperm from the two males do not meet, they cannot compete. Sexual interference in *T. vulgaris* is also discussed by Halliday & Verrell (1984). They propose that it could lead to 'internal' sperm competition; I feel that the qualification is unnecessary as sperm competition would have to be internal in any species where fertilization occurs internally.

Walker (1980) pointed out that, in insects, the shape of the spermatheca may influence the nature and extent of sperm competition. This may also be the case in urodeles and certainly more research needs to be directed at this area; Verrell & Sever (1986) have discussed female cloacal anatomy in *T. vulgaris*. Females have a spherical shaped spermatheca, which, according to Walker (1980), may suggest that the sperm from different males mixes freely. This is in contrast with the existence of some kind of sperm order effect which is thought to be suggested by a different shaped spermatheca.

Other reviews have considered sperm competition from a female perspective and considered the importance of the phenomenon to females (Parker 1984; Knowlton & Greenwell 1984). More recently, the increase in research looking into the mechanisms of sperm competition, e.g. sperm precedence and how it is effected, has been reviewed (Birkhead & Hunter 1990a). In a criticism of their approach in this review, Eberhard (1990) suggests that they have taken a male-oriented viewpoint and he emphasises the importance of the female perspective. However, as Birkhead & Hunter (1990b) point out in a reply to Eberhard (1990), although it is important to look at the evolution from a female viewpoint, this task will not be easy.

In this study, female sexual responsiveness was monitored over a breeding season. It was found that responsiveness could be very variable and this variation was documented. It appears that egg-laying is very important and poses a constraint on females responsiveness; egg-laying behaviour is itself constrained by temperature. Essentially this chapter has examined the adaptive significance of the observed variations in female sexual responsiveness and identified factors that affect it. Thus this study provides an ecological and functional context for the next chapter, which deals with female motivation from a causal perspective.

CHAPTER 5

Variations in Female Sexual Motivation - a causal viewpoint

5.1 Introduction

5.1.1 General Introduction

The preceding chapter discussed the longitudinal study of female sexual motivation from a functional viewpoint. An individual pattern of insemination was determined for each female and egg-laying behaviour was studied in relation to inseminations. The adaptive significance of these events was discussed in an ecological context.

This chapter deals with the same eight study animals as in Chapter 4, but takes an essentially causal perspective. However, the tests already described did not just consider whether or not insemination occurred; detailed observation of females' behaviour towards males was made during each test. The preliminary work in Chapter 3 provided the basis for this approach and the same fundamental aims apply to this work. However, certain problems were encountered in the preliminary study (Chapter 3), and the way in which they were dealt with requires some clarification here.

Section 4.2 explained how the females for the experiment (described in this and the previous chapter) were collected on migration to the pond. This eliminated one problem encountered in the preliminary study, which was that nothing was known about the history of the females when they were collected directly from the pond (i.e. how long they had been in the pond, whether they had previously mated or laid eggs, etc.).

Another problem with the preliminary study was the simplicity of the categorisation of female responses to males. A positive approach was the most receptive a response that could be scored with the system devised. The fact that so few successful courtships were seen contributed to the bias in the scoring system. In fact this system, in a sense, assumes that a female positive response to a male is a simple state (an assumption, indeed, that I have criticised earlier, Section 1.3.1). However, a

female that responds positively to a male and elicits the deposition of a single spermatophore may be less highly sexually motivated than a female that responds positively and then goes on to elicit three spermatophore depositions from a male. If a female picks up all of three spermatophores deposited by a male, she may be sexually more highly motivated than a female that elicits the deposition of three spermatophores, but only picks up one of them. It was evident that the scoring of female behaviour needed modification to take better account of the variability in female responsiveness and, consequently, measure sexual motivation more closely. This is dealt with in the results, presented in Section 5.4.

5.1.2 Brief Review of Descriptions of Female Smooth Newt Behaviour

A brief review of descriptions of female smooth newt behaviour in previous work is necessary here since they are few and are dispersed. The behavioural actions the female performs during a courtship encounter itself have already been described to some extent in Section 1.2.1. These have invariably been described simply in terms of how they relate to the male's behaviour and not as part of an interaction between two partners, each with an equal role.

Gauss (1961) describes aspects of female behaviour in detail as well as male behaviour but these are activities preceding the actual courtship itself. These have not been studied further as most subsequent work has focussed on the courtship encounter, and most often, on analyses of male behaviour.

In many species the male is more active in courtship than the female, who performs less complex or fewer behavioural patterns. In early studies of such sexual behaviour sequences, where there are few female activities in courtship, workers sought to analyse the sequence of male behaviour in isolation from that of the female. The idea was to explain links between male behavioural actions purely by processes internal to the male (e.g. Bastock & Manning 1955) although it was recognised to some extent that the female's behaviour may influence that of the male (e.g. Brown 1964).

An observational and experimental study of smooth newts (Halliday 1975) aimed to try and establish the causal relationships between male and female actions in the courtship sequence. The female was described as showing 'only three behaviours' (these were move away, remain still, and approach) (Halliday 1974; 1975), but she switched between them frequently (Halliday 1975). Because of this, Halliday (1975) believed it was not realistic to consider the male's behaviour in isolation, as had been done in the earlier studies mentioned above. Although this seems with hindsight to indicate a lack of awareness of the female's role in courtship, an appreciation of female behaviour has only recently been recognised as important (see Section 1.3.1 for discussion of this point).

Halliday used these three female behaviours to develop a 'strait-jacket' model, so that the female's behaviour in an encounter could be standardised (1975). The model consisted of an anaesthetised female in a small sleeve of flexible plastic fixed to a glass rod (Halliday 1975). The female is then under the experimenter's control of the and is a 'visually and olfactorially perfect model' (Halliday 1975).

The development of this model has undoubtedly enabled many facets of male behaviour to be more easily and thoroughly investigated. However, it also effectively precluded any further consideration of and investigation into the variation of female behaviour in courtship. No studies have dealt with female courtship specifically but aspects of it have been considered. Multiple insemination of females has been looked into (see Chapter 4 for a discussion). A component of female behaviour (continuous positive approach) has been incorporated into the NEWTSEX computer model, which aimed to simulate part of the male newt's courtship sequence (Houston et al 1977; Halliday & Houston 1991).

The only other aspect of female behaviour that has been discussed in previous work is whether or not she directs any behaviour towards the spermatophore. Tinbergen & Ter Pelkwijk (1938) and Arnold (1972) reported that a *Triturus* female lowers her cloaca upon a spermatophore or 'tactually' orientates towards it. In contrast, Halliday (1974) considers that the female shows no interest in the

spermatophore at any stage in the sequence. Also, Raxworthy (1989) states that it is quite clear that the female takes no notice of the spermatophore and makes no attempt to orientate towards it. In the present study I found that, at least on some occasions, the female was clearly seen to lower her pelvis and thus cloaca as she passed over the spermatophore. Previous workers may have missed observing this, especially if they were primarily concerned with observing, recording and timing male behaviour (e.g. Raxworthy 1989).

In summary, few details are known about the female's behaviour patterns and her role in the courtship sequence. This chapter aims to investigate these in detail and hopes to reexamine newt sexual behaviour in the light of what is found.

5.2 Aims

The two main aims for this study are:

1. To carefully observe female behaviour and draw up a detailed list of behaviour patterns in courtship, similar to that devised for males by Halliday (1974). Dawkins (1983) proposes ways of constructing ethograms. Those ideas have been followed to some extent in this study, in trying to pinpoint unvarying actions and define them.

The definitions of male smooth newt behaviour (Halliday 1974) have been used repeatedly in subsequent studies. They have also been successfully employed to help describe male courtship display in other *Triturus* species, although care must be taken to use the definitions accurately. This illustrates how fundamentally useful careful definitions of behaviour patterns can be for facilitating further research. This study aimed to produce a useful list of female behaviour patterns in courtship and use them to further investigate female responsiveness.

2. The second aim was to expand upon and improve the work described in the preliminary study (Chapter 3). In the study described in this chapter, the aim was to monitor the sexual motivation of individual females over the whole breeding season, not just a part of it. As before (see Section 3.2), females were presented

regularly with a randomly chosen male. Their sexual motivation was then measured by scoring their behavioural responses to the males' presence and courtship.

5.3 Materials and Methods

The eight females and the protocol used to test them were the same as those described in the previous chapter. As explained in Section 4.3, the females were presented with a randomly selected male every other day and the outcome of the pairing noted. In addition, for this part of the study, detailed descriptions of female and male behaviour patterns during each courtship encounter were recorded using a tape recorder and stopwatch (see also Section 2.3). These detailed observations were then used to fulfil the aims of this chapter. The males were also measured in order to investigate aspects of male morphology and mate choice (see Section 6.2).

As in the preliminary study (Chapter 3), it was hoped that the male's behaviour could be standardised as far as possible by allowing him to attempt to initiate courtship five times (see Section 3.2.3). A good stock of males was maintained to try and avoid the problems encountered in the preliminary study with reluctant males (see Section 3.2.3). However, males still became less ready to court as the season progressed; this phenomenon is discussed later (Section 5.4.2).

5.4 Results

5.4.1 Definitions of female behaviour

The female's behaviour is not elaborate like the male's and the actual behaviour she performs may not apparently be different at changing stages in the courtship sequence, to human perception at least. However, an approach by the female towards a fanning male may be in response to cues different from those available to her when she approaches a male that is depositing a spermatophore. In this way, the female has a sequence of behavioural actions in courtship, just as the male has. The courtship can, then, be described in terms of the female's activities, similar to the way in which it is more usually described in terms of the male's.

The female's behaviour patterns can be divided into two groups: phase 1 and phase 2. Phase 1 includes activities the female may perform before the male begins any of his courtship. These behaviour patterns could be termed 'proceptive' (Beach 1976) but in female newts (rather than mammals) they indicate a tendency to participate in courtship, not necessarily mating itself. Using the term proceptive is somewhat helpful in that it categorises a set of behaviour patterns that have been hitherto unrecognised or assumed to be very infrequent (e.g. Halliday 1974). However, a more neutral term is preferred here (phase 1) since it has none of the preconceptions associated with the earlier use of a term, which can often confound clarity.

Phase 2 includes all those behavioural actions performed by the female after the initiation of courtship by the male. The details of these two phases are described below; details of male behaviour patterns used below have been discussed earlier (Section 1.2.1).

Phase 1:

Distant approach: female approaches male to a distance of $>$ one body length.

Close approach: female approaches male to a distance of $<$ one body length.

Sniff (earlier defined in Marquenie {1950}; Halliday {1974}): whilst $<$ one body length from the male, female flexes her buccal surface, indicating she is sampling the water or 'sniffing' close to the male.

Phase 2:

Stand: female remains motionless after the male has performed a move-to-the-front display. He then begins his static display.

Static display approach (roughly equates to 'positive approach', Raxworthy {1989}): female continually approaches male while he is performing static display. He usually enters retreat display.

Retreat display follow: female consistently follows the male while he is performing retreat display. He usually turns away from the female into creep, beginning the sperm transfer phase of the courtship.

Creep follow: female follows the creeping male. He usually stops after a few seconds to quiver.

Tail touch (Halliday 1974): female touches the male's quivering tail with her snout. This stimulus is usually required to elicit spermatophore deposition from the male.

Creep-on follow: female follows male that is creeping-on after spermatophore deposition.

Pick up: female picks up the sperm mass in her cloaca, removing it from the spermatophore base.

Brake approach: female approaches the male after he has turned through 90° into brake position.

Brake touch (this has also been referred to as the second tail-touch (Halliday 1974; Green 1991)): female's snout touches the male's folded round tail in brake position. The definition given here is preferred because it avoids possible confusion with the tail touch (above) that elicits spermatophore deposition.

Fig 5.1 incorporates these new terms to show smooth newt courtship from a female perspective. At any stage in the courtship, the female may move away from the male, but this becomes less likely as she progresses through her courtship sequence. It may, perhaps, seem unnecessary to look at the courtship in this way when it has already been described adequately enough for most purposes (Halliday 1974). However, the perspective illustrated in Fig 5.1 is considered valuable for two main reasons. Firstly, it underlines the specific role the female plays in courtship and, secondly, it emphasises how the sequence of behaviour patterns of each sex interacts closely with that of the other. In addition, definition of the female's behaviour patterns enables previously unconsidered aspects of the courtship to be investigated in an objective and quantifiable manner (see Section 5.4.4).

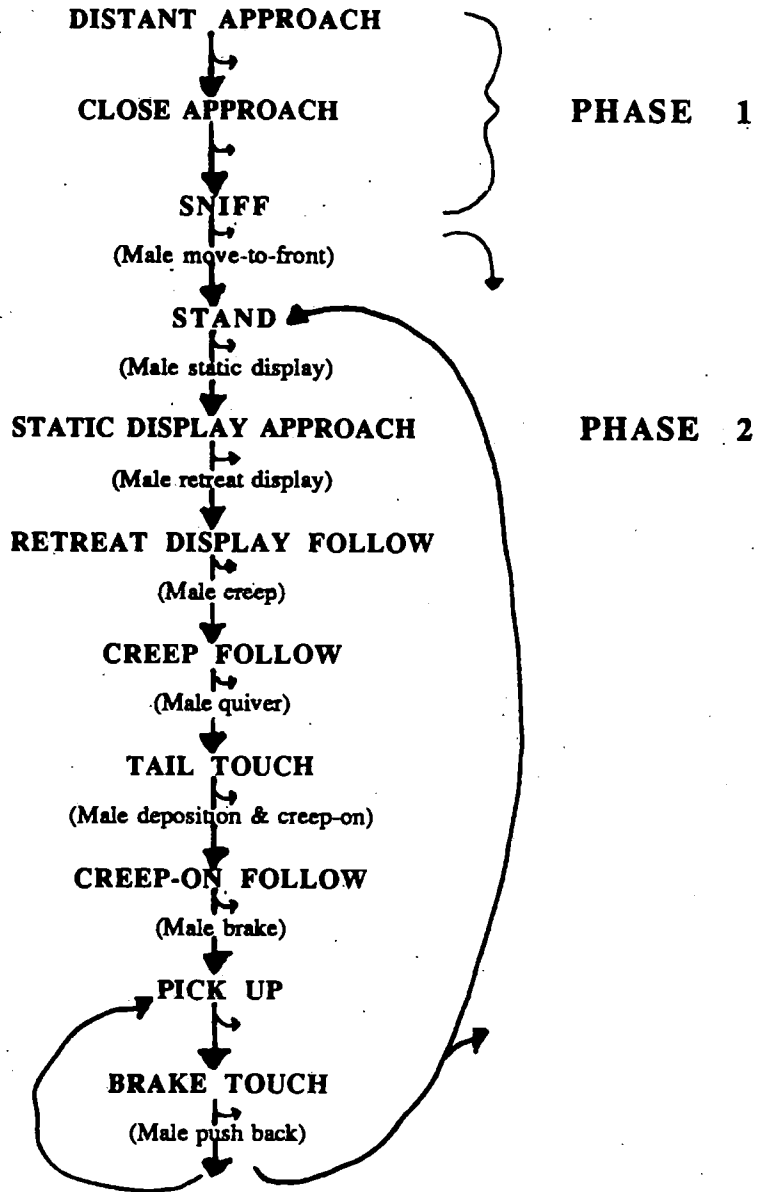


Fig. 5.1 Schematic diagram showing the females' sequence of courtship behaviour patterns. The side pointing arrow at each step indicates that the female may move away from the male at any stage in the courtship. If this happens courtship may be resumed at the beginning of phase one, or more usually, of phase two.

5.4.2 Scoring sexual motivation

It was pointed out in Section 5.1 that the scoring system used in the preliminary study would need modification before being used here, to take better account of the variability in female responsiveness.

The motivation score that each female received for each test over the season consisted of two parts. These were directly related to the definitions of female behaviour patterns shown in the previous section. When the male was present in the test tank but had not yet begun any courtship, the female sometimes performed some or all of the phase 1 behaviour patterns described above. As the phase 1 part of her motivation score, she received one point for performing any of these. To try and keep things reasonably simple, the number of times any of these behaviour patterns was performed was deemed unimportant, just whether they were or not. So if the female performed at least one of each of the three behaviour patterns: distant approach, close approach and sniff, she received the maximum phase 1 score of three, one point for the performance of each.

To compile the phase 2 part of the motivation score, one point was given for each of the behaviour patterns performed in the sequence outlined in the above section and Fig 5.1. A maximum of five move-to-the-fronts was allowed but five were not specifically required (see below). This is basically straightforward but a number of points need clarification.

Each encounter was allowed to progress without any interference. Once courtship had begun, it was allowed to continue until the female ceased to participate and showed no further interest in courting. For each spermatophore deposition, the sequence of female behaviour patterns (Section 5.4.1) was usually exhibited. The female scored one point for each behaviour pattern performed, in each separate sequence. However, only one point was given, if the behaviour pattern was shown twice in the same sequence. For example, if the female performed 'stand' three times before going on to 'static display approach', she would only score one point for stand.

An example should make this clearer. Consider a female that elicits the deposition of two spermatophores and picks up the first, but not the second of these. She then walks away after 'brake touch' and shows no further interest in courtship. This female would score 9 for the first sequence and 8 for the second, making the total phase 2 motivation score 17. If, earlier in the test, this female had received a phase 1 score of 2, her total motivation score would be 19 for that test. In this way, a motivation score was compiled for each female, in each of the 33 test encounters staged over the season.

While still less than perfect, this scoring system is considered an improvement on the earlier one used in the preliminary study (Chapter 3). The new system takes full account of the number of spermatophore deposition sequences performed in an encounter, and indeed, the number of spermatophores picked up. This is, in fact, where most of the variation in courtship encounters lies, not in whether or not courtship progresses to the deposition phase, which is where the emphasis of the previous system lay.

The problems with hoping that males would perform a standard amount of courtship (five move-to-the-fronts) have already been mentioned. Male reluctance to court has been encountered with freshly caught animals and with those kept in the laboratory or outside shed for varying periods of time (pers. obs.). Verrell (1986b) found evidence for male choice for larger females, so this male choosiness may be a confounding problem here. The new scoring system minimises the effect of this problem since it does not require a response to five move-to-the-fronts by the male; it allows courtship to take place in a more natural fashion.

5.4.3 Motivation profiles

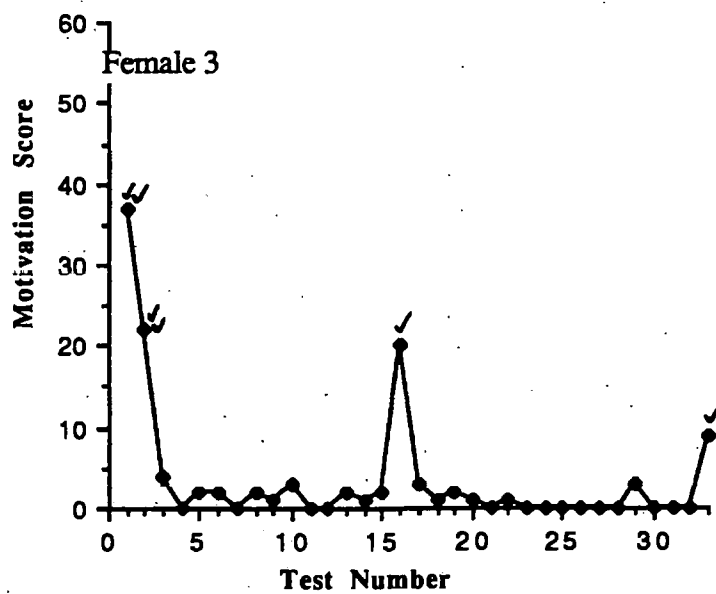
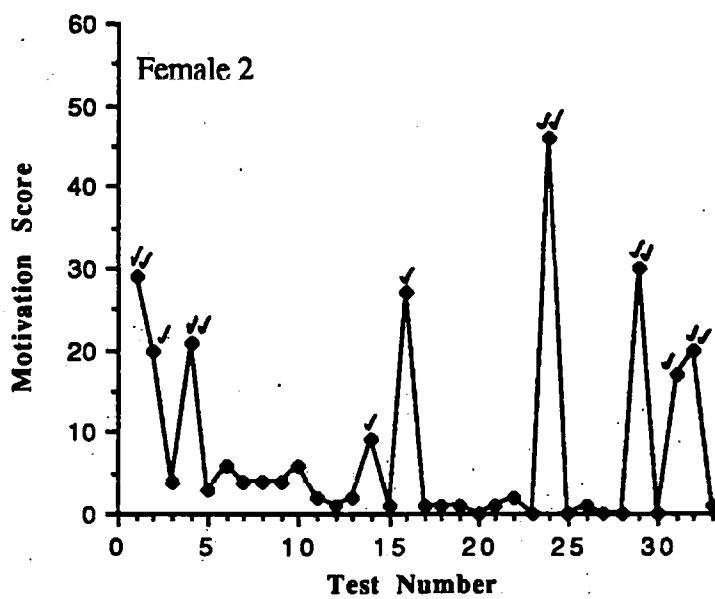
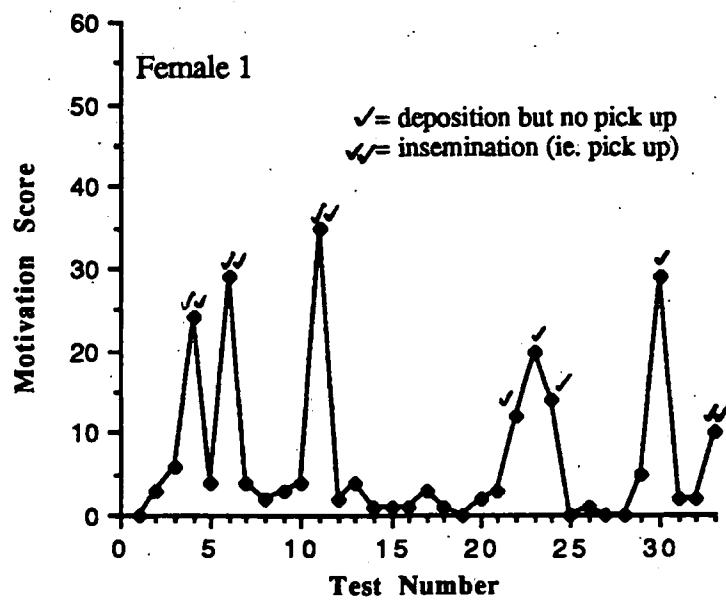
The method for deriving a motivation score has been described above. At the end of the season, each female had a motivation score for each test. In most cases the end was test 33, but some females became terrestrial earlier than others and testing for these obviously ceased earlier. All the motivation scores for each female were plotted

sequentially to give a motivation profile for the season. These profiles are shown in Fig 5.2 for the eight females tested here.

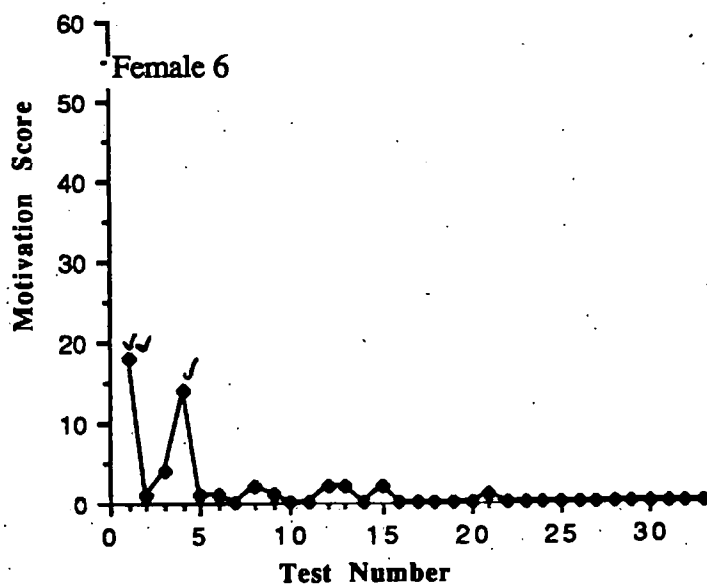
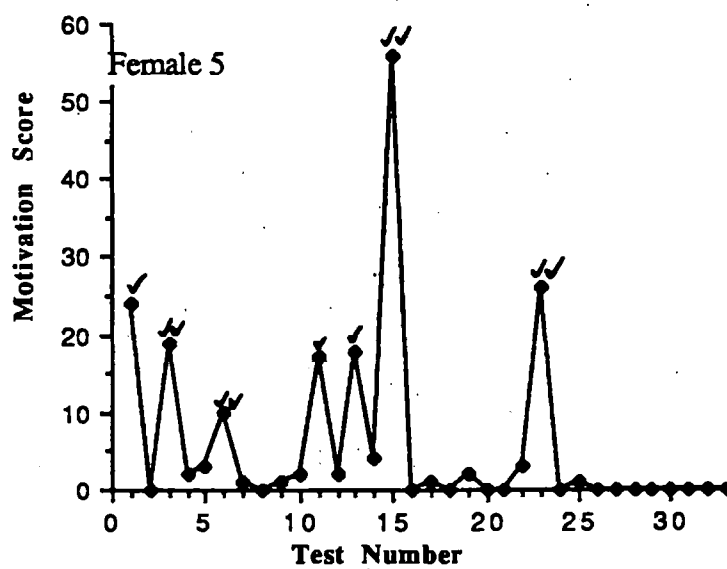
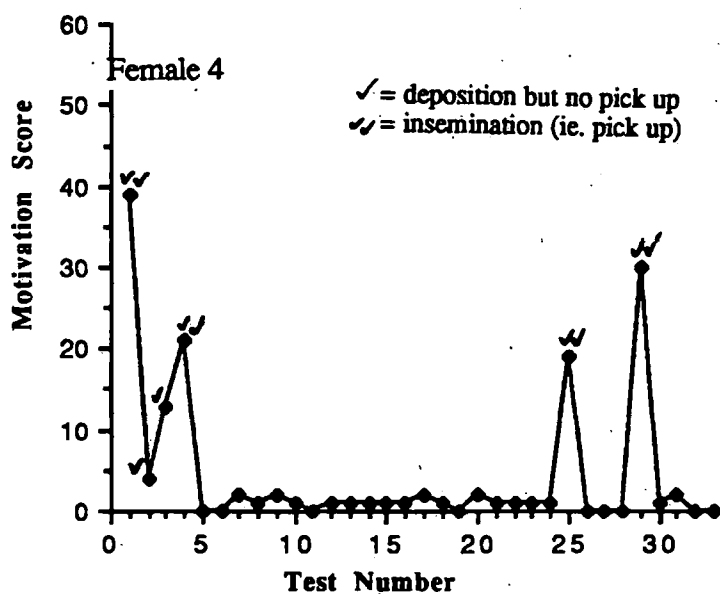
A number of general points emerged from studying these motivation profiles. There seemed to be no evidence for any kind of pattern of motivational change over the season. The only aspect that appeared constant between different females was the high level of insemination in the first six tests. (This has already been discussed in Chapter 4, Section 4.4.1.) What is of more interest here is the varying levels of sexual motivation the females appear to attain. The motivation scores that females achieve when insemination occurs (see Fig 5.2) are very variable, ranging between 9 and 53. The variation reflects, to some degree, the different numbers of deposition sequences that occurred in tests.

In Chapter 4 (Section 4.4.1) the season was divided into two parts, 'early' and 'later'. This division was also used here since it essentially forms part of the same experiment. The motivation scores for tests where insemination occurred were compared between the early (mean=23.6) and later (mean=25.7) parts of the season. There was no significant difference (Student's t-test; $p=0.6$) between them. A further comparison, that of motivation scores for encounters that result in deposition only (no pick up: early mean=17.0, later mean=17.1) and motivation scores for insemination (deposition and pick up), was also not significant (t-test). This was found to be the case for both the early ($p=0.06$) and later ($p=0.07$) parts of the season. This may indicate that there was nothing about the female's behaviour that reflected the outcome of an encounter involving spermatophore deposition; i.e. whether or not the female became inseminated.

These results suggest that there may have been some threshold level of motivation which females reached when they performed retreat display follow, which indicated their readiness to go on to the spermatophore transfer part of courtship and thus potentially become inseminated. The results also indicate that this threshold did not change over the course of the season. These issues are returned to in the final chapter, where they are discussed in the context of male morphology and mate choice.



continued



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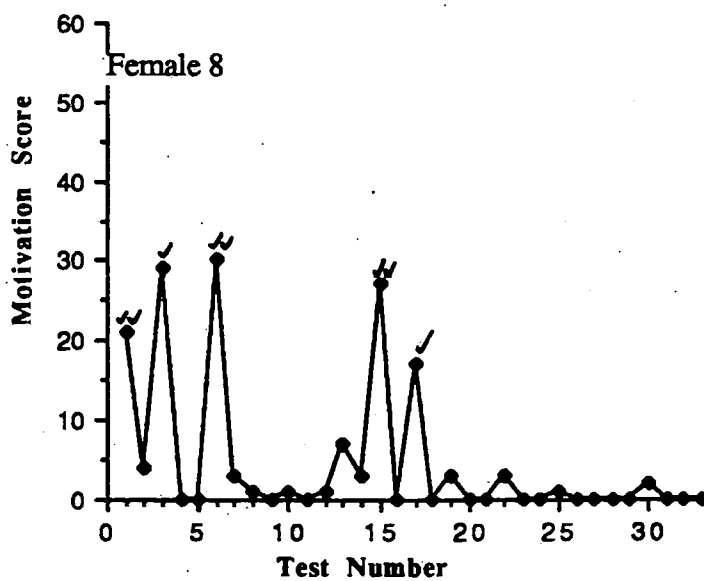
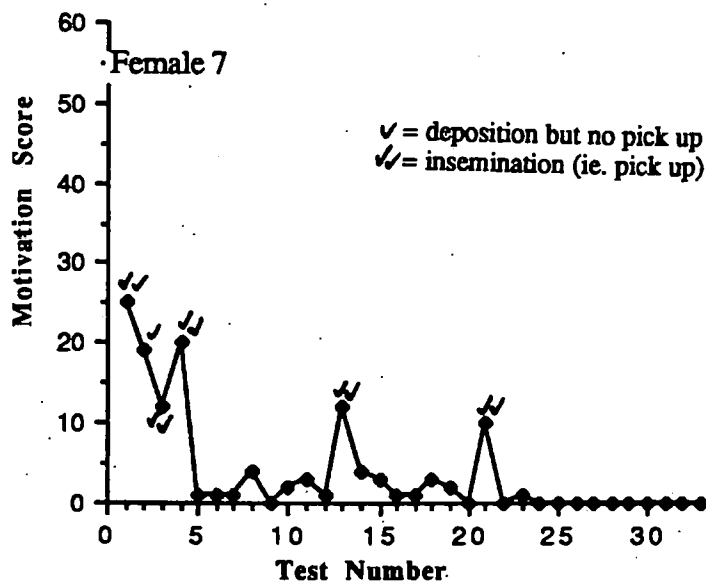


Fig. 5.2 Motivation profiles for the eight test females over a whole breeding season (33 tests). (See text for details).

This section has described an attempt to measure female sexual motivation reasonably objectively and has charted it (for eight individual females) over a breeding season. What is of further interest is a consideration of the factors that contribute to the female's sexual motivation. The next section investigates this more closely.

5.4.4 Further investigation of female responsiveness

The development of a catalogue of female courtship behaviour patterns has enabled a scoring system for female sexual motivation to be devised and used (see above section). The catalogue of female behaviour patterns can also be used to look at female responsiveness in more detail.

A female's sexual motivation is likely to be made up of different components. Both her internal state and external stimuli will contribute in some way to these different components. It is likely that, over an extended breeding season, the importance of these different components of female sexual motivation may vary. This was investigated in this chapter, and compared between the early and later parts of the season, as follows.

It is important to recall that, for those encounters where insemination occurred, there was no significant difference between the motivation scores for the early and later parts of the season. The level of sexual motivation necessary for insemination did not change over the season, but did the different factors that made up that motivation vary?

The male's display has been shown to alter the female's receptivity and to influence the likelihood of insemination occurring (Teyssedre & Halliday 1986). The female's phase 1 behaviour patterns are performed before the male begins any static display, but in his presence, so the opportunity for courtship and mating exists. It is proposed here that the performance of these behaviour patterns can be taken as a reflection of the level of female sexual motivation prior to exposure to male display. This is termed 'pre-display responsiveness'. It refers to the level of the female's

sexual motivation at the beginning of a courtship encounter, which results from all factors other than the male's display.

Once male display begins it may influence a female's sexual motivation. This is termed 'display responsiveness' and it refers to the contribution to the female's sexual motivation made by male display. At the point in courtship when a female follows a male consistently in retreat display ('retreat display follow') she indicates she is ready to move on to the spermatophore deposition phase of courtship and, thus, potentially become inseminated. The length of time that a female allows a male to display to her before she performs retreat display follow is proposed, then, to be a reflection of her display responsiveness. This length of time is called *total stand time* for convenience.

For the present purpose, the level of female sexual motivation required for the female to perform retreat display follow is considered to be made up of two components: pre-display responsiveness and display responsiveness. The level of performance of phase 1 behaviour patterns is taken to reflect a female's pre-display responsiveness and her total stand time is taken to reflect her display responsiveness. The definitions of female courtship behaviour patterns allow these aspects of her courtship sequence to be quantified and investigated.

It was thought that the importance of phase 1 behaviour patterns in courtship may be different at different stages in the season. To obtain some idea of this, the proportion of courtship encounters that had a maximum phase 1 score was compared between the early and later parts of the season (see Fig 5.3). A Wilcoxon test was used to compare this for each female under the two conditions, early and later. The comparison was highly significant ($p < 0.01$) indicating that phase 1 behaviour patterns feature much more strongly in courtship in the early part of the season than in the later part. This indicates that pre-display responsiveness is an important part of female sexual motivation in the early part of the season but less important in the later part.

To investigate display responsiveness, the total stand time for each encounter that progressed to retreat display follow was calculated. The mean total stand time was

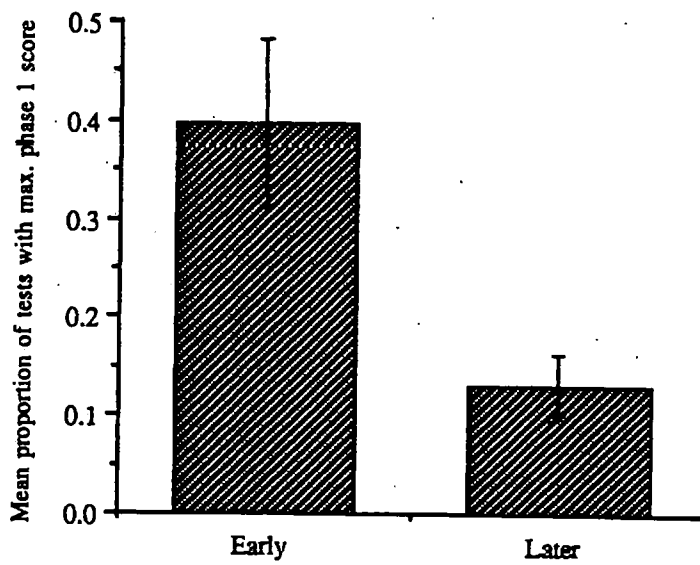


Fig 5.3 Mean proportion of tests with a maximum phase one score; shown for early and later parts of the season. (See text for details).

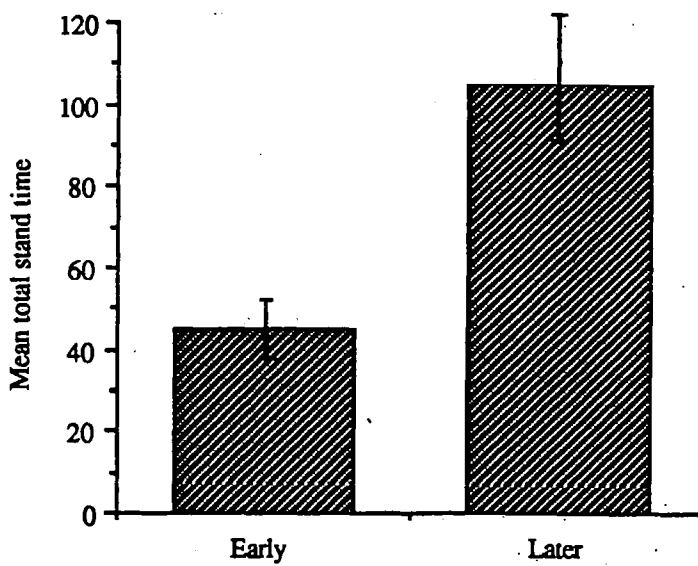


Fig. 5.4 Mean total stand time; shown for early and later parts of the season. (See text for details).

compared between early and later (see Fig 5.4) for each female (Wilcoxon test). This showed a highly significant difference ($p < 0.01$); thus females allowed males to display to them for much longer periods of time in the later part of the season than in the early part. This indicates that the female's display responsiveness is a more important part of female sexual motivation later in the season than it is in the early part.

These results suggest that the components that comprise high female sexual motivation vary in importance at different stages in the season. Early on, the factors that are unrelated to male courtship appear to be of greatest importance and females seem more ready to mate at the beginning of a courtship encounter (see also Chapter 4). Later in the season male courtship display appears to be of greater importance for females in attaining a high sexual motivation. The important relevance of this to mate choice studies is discussed in the following chapter.

5.4.5 Heterotypical behaviour

During the course of many hours of observation in the preliminary study (Chapter 3) and the work discussed here, females were seen to perform heterotypical behaviour (Haug et al 1990). That is, they performed behaviour patterns that would normally be described as belonging to the male's repertoire. In this study females were seen to perform fan, wave and whip, and retreat display. Heterotypical behaviour was also noted by Halliday (1974) but referred to as pseudomale behaviour.

In the preliminary study (Chapter 3) it was performed at some time by five out of the eight females, three performed it once only and two more than once. In the present study, six of the eight females performed it at some time. Two females once only over the whole season, one female in two tests, two in three tests and female eight performed it in seven tests.

Why females might perform these behaviour patterns is somewhat puzzling. In some species it is quite normal for one sex to perform heterotypical behaviour (e.g. all-female lizards [*Cnemidophorus uniparens*], Moore et al (1985); a mourning

gecko (*Lepidodactylus lugubris*), McCoid & Hensley (1991)). In female smooth newts it is difficult to discern a function (if one exists) for this behaviour.

It has all the hallmarks of a displacement activity (Timbergen 1951): it looks rather odd and appears irrelevant. However, as Dawkins (1986) points out and discusses, some behaviour patterns that appear irrelevant may have a real function but it is simply not obvious to a human observer.

In this study, heterotypical behaviour occurred eight times in the early part of the season, and nine times over the whole of the later part. It does seem that its performance is associated with a high sexual motivation. In 11 out of the 17 incidences, spermatophore deposition also took place and, in eight of these, insemination occurred. Interestingly, four of these insemination encounters were also occasions when large numbers of spermatophores were deposited (4, 4, 5, 6). On only five occasions in the whole of the present study (this chapter) were four or more spermatophores deposited; heterotypical behaviour also occurred in four of them.

Morris (1955), Halliday (1974) and Hinde (1970) all suggest its performance may be associated with sexual 'deprivation'. Certainly this study suggests it occurs when the female is highly sexually motivated but whether it has any particular function awaits further study.

5.5 Discussion

This chapter discusses the result of detailed observations of courtship behaviour from a female perspective. From these, an idea of the female's specific role in courtship has been obtained and a sequence of behaviour patterns has been derived. The behaviour patterns that the female performs prior to any male display have been emphasized to underline the importance of this aspect of courtship. It is not unusual for females to approach males and initiate courtship (cf. Halliday 1974). From the work discussed here and in Chapter 4 (also from pers. obs. in subsequent years), it is evident that it is quite normal for females to be highly sexually responsive before male courtship begins.

This is especially true at the beginning of the breeding season before egg-laying has really begun (see Section 5.4.4). Since more inseminations occurred in the early part of the season than in the later part (see Section 4.4.1), it could be argued that it is at least as 'normal' for females to be responsive at the start of courtship as for them to be generally unresponsive. Previous studies, where females were collected from the pond after the beginning of the breeding season, would not have been able to detect this. Only after monitoring females over a whole season has this wide variation in female responsiveness come to light.

The phase 1 and phase 2 behaviour pattern definitions have been used to devise a scoring system for female sexual motivation. This is more meaningful than the previous attempt to measure sexual motivation (see Chapter 3) since it reflects better the natural variation in female courtship behaviour. Although it is still somewhat crude, it has enabled motivation profiles to be drawn up for individuals over a whole season and female responsiveness to be further investigated.

Fig. 3.4 (in Chapter 3) showed a model of female responsiveness, modified from the original hypothesis (Section 3.1.1), in the light of the results of the preliminary study (Chapter 3). Fig. 5.5 extends and clarifies the model further, and summarises the results found in this chapter.

It has been demonstrated that female sexual responsiveness is a rather complex phenomenon. One important practical point that arises from this is the use of the strait-jacket female model in experiments. It has often been noted (pers. obs. & Waights per. comm.) in tests involving these that males sometimes appear to be 'wary' of the 'females' behaviour or not respond at all. (Most men know that there is something suspect about an apparently perfect female!) Perhaps the results presented here will enable a more naturally-behaving model to be devised, which would extend its usefulness still further.

Insemination is the culmination of two closely interacting processes, each of which depends on the internal state of the individual, and the interaction of internal and external stimuli. No two courtship encounters are ever likely to be the same in these

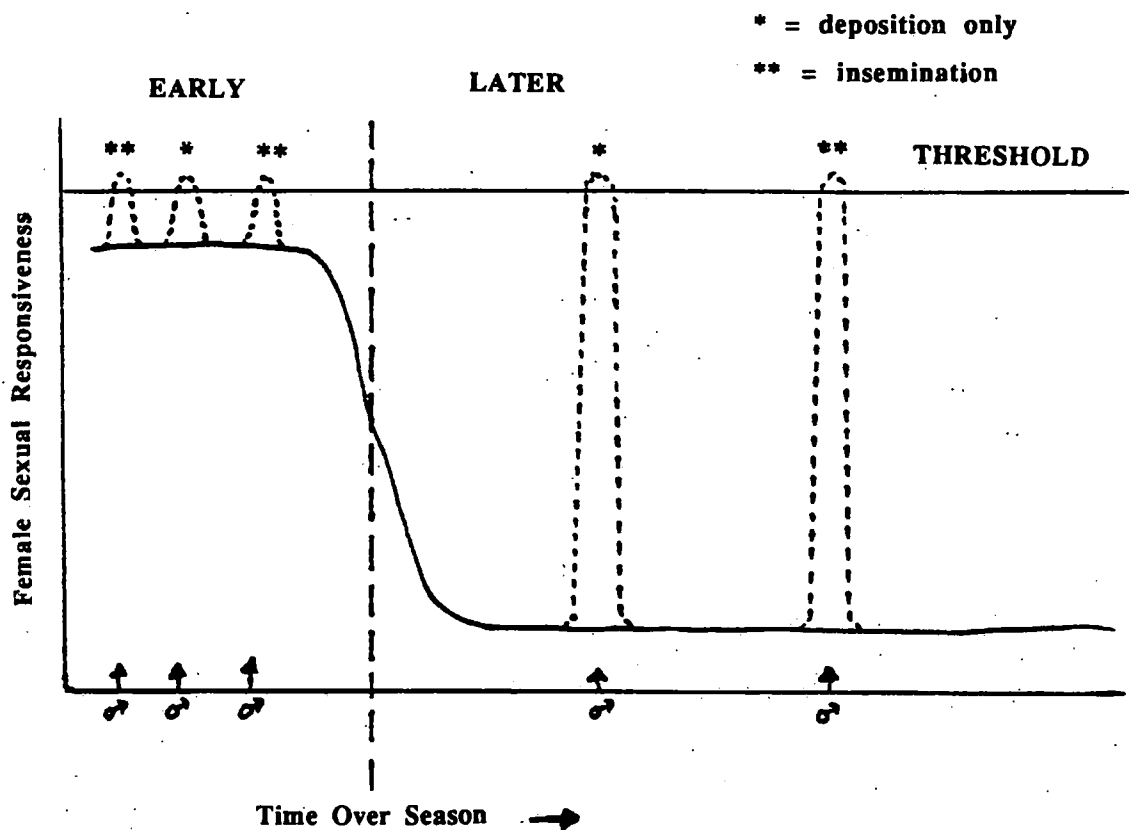


Fig. 5.5 Modified model of female sexual responsiveness. The threshold indicates the level of responsiveness when the female enters retreat display follow. The arrows and male symbols indicate incidences of courtship display. The solid curve represents the female's pre-display responsiveness; the dotted curves represent her display responsiveness. (See text for details.)

respects, but some of the components that make up sexual motivation can be identified. How the different components of female sexual motivation vary over a season has been studied in this chapter. Male sexual motivation is evidently also a complex phenomenon (see Halliday 1976, for a study of male 'libido') and this has contributed to problems with trying to obtain standard amounts of courtship from males.

The work on modelling male courtship behaviour (Houston et al 1977; Halliday & Houston 1991) could now be extended to include a more natural female component. Better still, an attempt could be made to model female courtship and an interactive model of courtship could be devised. Future work will follow this up.

No attempt has been made in this study to investigate the mechanisms that might underlie female sexual responsiveness. Some possible mechanisms were briefly discussed in Section 4.5 with regard to multiple mating. However, until more details are known about the female's physiology, only tentative hypotheses can be suggested.

The 'black box' behavioural approach (Section 1.3.3) has been used here, with some success, to look at female sexual motivation from a causal perspective. With this work as a background, the following chapter goes on to look at mate choice in female smooth newts. The final chapter brings together a discussion of the different approaches taken here in studying motivation and how these are important to the study of mate choice.

CHAPTER 6

Aspects of Mate Choice

6.1 Introduction

As has already been mentioned (Section 1.3.5) the marked sexual dimorphism this species possesses makes it an interesting subject in which to investigate sexual selection by female choice. At the time of performing these experiments I was unaware of any studies that had been done (for smooth newts) on the important question (see below) of whether males that successfully inseminate females have larger crests than males that do not. Studies on the crested newts, *T. carnifex* (Malacarne & Cortassa 1983) and *T. cristatus* (Hedlund 1990) give some evidence for discrimination for higher crests in these species.

The link between the extent of development of a male's secondary sexual characteristic and his condition has already been touched upon in Section 1.1.3. It is particularly pertinent for study and discussion in the genus *Triturus* since many species have such marked dimorphism. In addition, recent work has begun to illuminate these relationships in different *Triturus* species. A study by Baker (1992) showed, for *T. cristatus*, a correlation between weight condition on arrival at the breeding pond and tail height developed later in the season.

A similar laboratory study on smooth newts (Green 1991) showed a correlation between food intake (over the twelve days prior to measuring) and crest height. That study also reported a correlation between crest height and condition in the field: for a given snout-vent length, heavier males had higher tails.

Green's (1991) study is interesting because it relates recent foraging intake with crest height and he concludes that crest height is an honest indicator of phenotypic vigour. Baker's (1992) study adds a further dimension, as the relationship he found was essentially between foraging intake during the terrestrial phase and later crest development. Since an individual's foraging success in the terrestrial phase may also determine the number of gametes he can mature, the idea that the crest may be a

'viability indicator' and important in female choice (Andersson 1982; 1986) becomes even more compelling. This idea is investigated by some of the work described in this chapter.

Studies that have directly tried to investigate mate choice in smooth newts will be briefly reviewed here, to provide a context and background for the work that was carried out as part of the present project. First, however, the definition of mate choice that is used and understood in this work must be clarified.

Halliday (1983a) operationally defines mate choice 'as any pattern of behaviour, shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others'. Halliday notes that this definition is in terms of observable behaviour and covers a wide variety of phenomena that might not generally be called choice. What may be observed is selective responsiveness to particular stimuli and the mechanism whereby this is effected is irrelevant. What is important in evolutionary terms is simply whether variations in the behaviour of members of one sex are correlated with variations in their mating success (Halliday 1983a).

The phenomenon of mate choice in smooth newts is more complex than might first be imagined and previous chapters have shown that it can be confounded by female motivation (see Chapters 3 and 5) and other issues such as multiple mating and sperm competition (see Chapter 4).

During the course of a study of sexual interference between courting males, Verrell (1983) observed that female newts seem to find these competitive sexual encounters 'aversive'. They would often move away when the second male approached (Verrell 1983). This is interesting from a mate choice perspective because it appears that females find aversive the very situation in which they could potentially compare two males, at least in terms of secondary sexual characteristics and/or courtship display.

A further study examined this aversion more closely. Verrell (1984c), using proximity as an indicator of preference, looked at females' responses to different

densities of males. The study found that females spent significantly more time in the vicinity of one male relative to four males, one male relative to three males and one male relative to an empty box. Verrell (1984c) concluded that female smooth newts in breeding condition find conspecific males visually attractive but only if they are present one at a time. These studies have looked then at male density, but not at any aspects of the crest height of the males involved.

Verrell's (1983) study also discussed the possibility of assortative mating in this species. This is when mating occurs between certain phenotypes more often than expected under random mating (Partridge 1983). In newts this could take the form of individuals of similar snout-vent length mating with each other. This confers a potential advantage in sperm transfer since, during courtship, when the male has deposited his spermatophore, he moves through a distance approximately equal to his snout-vent length, before turning into brake position (see Section 1.2.1). Brake halts the further progress of the approaching female. If she has a similar snout-vent length to that of the male she may be more likely to stop with her cloaca over the spermatophore than if she is much larger or smaller than him.

An earlier study by Halliday (1983a) found that females tended to pick up later spermatophores in a sequence. He suggests that this reflects a subtle form of mate choice since by doing this, the female is favouring more fertile males, i.e. those able to put down more spermatophores.

The work discussed in this chapter has taken different approaches to investigating mate choice. It is quite likely that there is not just one simple rule whereby females 'decide' on a mate. Indeed, it may also be confounded by the choosiness of the males (Verrell 1986b for *T. vulgaris* and see Verrell 1985, for a discussion of this phenomenon in *Notophthalmus viridescens*). It was hoped that, by taking different approaches to investigating mate choice, a broader understanding of the processes involved might be achieved than with a single approach.

6.2 Aims

1. The first aim was to look at whether any evidence for size assortative mating existed for pairs (one male, one female) chosen at random and allowed to court. After the pairs had been put together their snout-vent length's (SVL's) and the tail heights (THs) of the males were recorded. The SVL data allowed assortative mating with respect to body size to be investigated and the TH data were used in 2 below.

2. The second aim was to see if males that actually inseminate females have larger crests than those that do not. Tail height was used as a measure of crest height since the two scores are closely correlated but tail height is much easier to measure accurately (Baker 1990b).

This method of simply pairing two individuals randomly with each other obviously does not give females the opportunity for direct comparison between two mates. However, as it is not known how individuals encounter and interact with each other in the wild, and as females seem to generally find more than one male aversive (Verrell 1983), it was considered a valid way in which to investigate female choice. It also complies with the definition of mate choice given in Section 6.1, as do all the aims of this chapter.

3. Halliday (1983a) interpreted the order of pick up of spermatophores as a subtle form of female choice. To further investigate this, the orders of pick up of the spermatophores deposited in the above experiment (aims 1 and 2) were examined, to see if Halliday's (1983a) results were supported.

These were the main aims for this part of the project and were carried out in 1989 (they are referred to as the mate choice experiment). In addition, these three aims were investigated in the 1989 motivation experiments (see Chapters 4 and 5), since (as noted in Section 5.3) tail height and spermatophore data were recorded for each of these tests with a view to looking at aspects of male morphology and mate choice. As well as completing these three aims in 1989, other aspects of female choice were studied in the following year. The aims for this work are detailed below, 4 and 5.

4. Since for whatever reason(s) females multiply mate, there may be an adaptive reason for showing a different response to familiar and unfamiliar males, especially at the beginning of the season. It may be adaptive for a female that has been inseminated by one particular male not to mate with him again. For example if he happened to be infertile, it would clearly be disadvantageous to mate with him twice, rather than mating with a different male the second time. In this experiment females were given the opportunity to mate once and then given a second opportunity. At this point, half were presented with the same male and the rest with a different male. In this way female responses to familiar and unfamiliar males could be examined.

5. The final aim was to investigate the responses of unmated females to a simultaneous choice situation. The earlier study involving two males and a single female (Verrell 1983) used females collected from the pond, after the beginning of the breeding season. Earlier work in this study (see Chapters 4 and 5) showed that females tend to be more receptive at the beginning of the breeding season. Thus, in this experiment, unmated females were presented with a large-crested and a small-crested male at the same time to see if, (a) they find the situation aversive and, (b) if not, whether they exercise any obvious discrimination between the two males.

This develops Verrell's ideas (1983; 1984c) by further investigating the effect on the female of the presence of two males and also providing the female with males with different sized crests.

6.3 Materials and Methods

Animals used for this mate choice experiment were aquatic and collected from Great Linford and Conniburrow ponds. General collection and maintenance methods were as described in Chapter 2. The experimental method involved for the first three aims is described in Section 2.3. The randomly chosen pairs were left together for an hour or, if they began courting, until courtship ceased.

The methods for aims 4 and 5 were essentially the same except that the two males with which the females were to be paired were not chosen at random, but were

as explained in Section 6.2 above (and see results for description of differences in tail heights for pairs of males).

All measurements were taken from unanaesthetized individuals gently restrained on a grid. SVL was measured from the tip of the snout to the distal point of the cloaca (see Fig 6.1). TH was measured as the greatest vertical depth of the tail blade (see Fig 6.1).

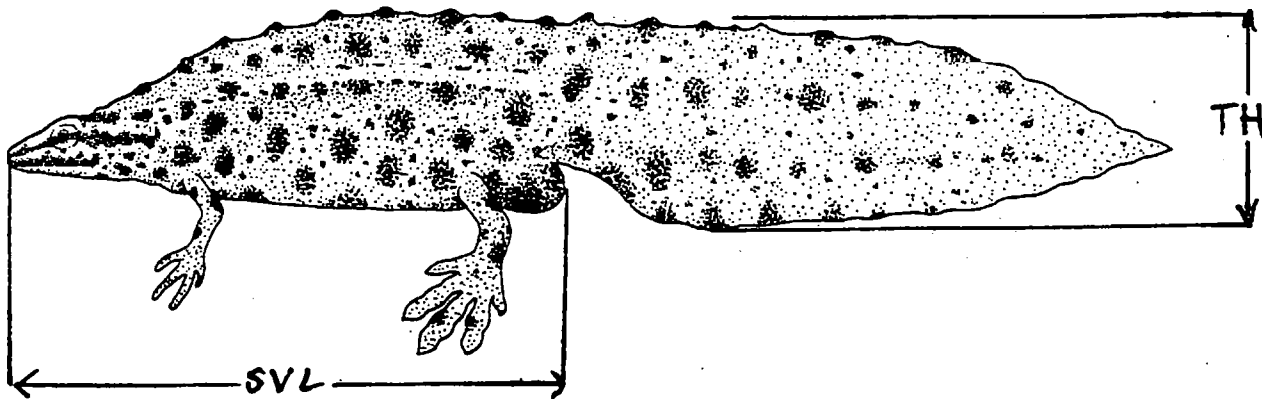


Fig. 6.1 Measurements taken from male newts used in mate choice tests. (See text for details.)

6.4 Results

6.4.1 1989 Mate Choice Experiments

A total of 105 pairs were put together to court. In 39 of these the male did not even attempt to court the female. In the remaining 66, at least some courtship was shown by the male towards the female. Of these encounters 12 culminated in spermatophore deposition (11% of total) and in only 8 was sperm actually picked up. Thus, despite the large number of pairs put together only a very small proportion (8%) actually achieved insemination. Since insemination was the criterion used to indicate

choice in these experiments, the sample sizes available for comparison are small, and contain much variation.

When the SVL data were examined, no correlation between male and female SVL was found for successful courtships (i.e. those where sperm was picked up by the female) (Spearman rank, $r=0.3$; $p=0.6$). The data for those encounters where no courtship occurred were also examined as it was thought that there may be a negative relationship between SVLs of males and females that showed no interest in each other; however, no such relationship was found (Spearman rank, $r=-0.15$; $p=0.5$).

The paucity of inseminations also meant that TH data for successful courtships were few. There was no significant difference (Student's t-test; $p=0.17$) in TH for successful males (mean=11.8mm; $n=8$) compared with unsuccessful (no deposition) males (mean=10.9mm; $n=43$). In addition, when THs of males that did not deposit a spermatophore were compared (t-test) with THs of all males that did deposit (whether it was picked up or not) (mean=10.9mm; $n=12$), there was also found to be no significant difference between them ($p=0.2$). These experiments were done quite late in the season, due to time constraints, and this may have contributed to the low insemination rate; this is discussed in Section 6.5.

6.4.2 Mate Choice In Motivation Experiments

The data from the experiments described in Chapters 4 and 5 will now be discussed. A large number of matings occurred during these experiments which resulted in a useful amount of data to investigate mate choice phenomena.

First the possibility of size-assortative mating was assessed. There was no significant correlation between male and female SVL for successful courtships. Thus, as was found with the data in the previous section, there is no evidence for size assortative mating.

The data on TH will now be discussed. To aid interpretation of this the final stages of the courtship will be very briefly redescribed from Section 1.2.1. After performing static display for some time the male may be approached by the female. He

turns into creep, stops, quivers and receives a tail touch from the female if she has followed him. This elicitation of spermatophore deposition is an important step in the courtship because once the spermatophore has been deposited, the potential for insemination exists. The next important point of variation in the courtship is whether the female follows the male such that she actually picks up the sperm and become inseminated. The two important female 'choice' points to bear in mind are whether the female elicits deposition and, if so, whether she picks up the sperm deposited.

The results for the comparisons of tail heights between different males are shown in Fig 6.2. Males that successfully inseminated females had significantly higher tails (mean=12.28mm;n=29) than unsuccessful (no deposition) males (mean=10.43mm;n=177) (Student's t-test; $p<0.001$). In addition, there appeared to be another level of discrimination between males by females: the tails of successful inseminators were also significantly higher (t-test; $p<0.05$) than those who achieved deposition but not pick up (mean=10.72mm;n=18) (see Fig 6.2).

These results show that, for this experiment, there was some discrimination in favour of males with deeper tails at both the deposition and pick-up stages. (The mechanism by which this discrimination might operate is discussed in Section 6.5.)

However, it was pointed out in Section 4.5 that females mated frequently in the very early part of the breeding season, indeed before males have been found to show maximal crest development (Griffiths and Mylotte 1988). In addition Section 4.5 noted that females seemed not to be particularly discriminating at this early stage in the breeding season.

In order to throw light on this interesting situation it was considered instructive to examine the data for the early and later parts of the season separately. In Section 4.4.3 (and Fig 4.3) a distinction was drawn between the time over which the first six tests were performed and the rest of the season. For the purposes of further discussion these two parts of the season will be referred to as 'early' and 'later' respectively.

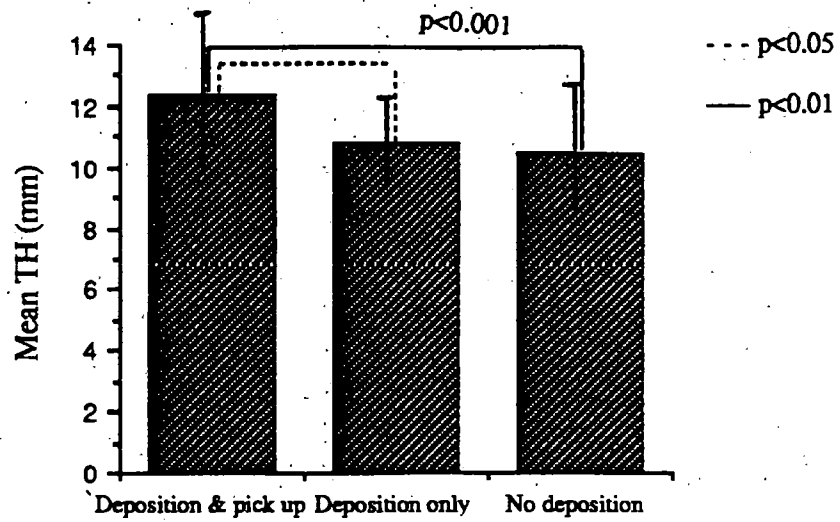


Fig. 6.2 Mean tail heights for males that achieved different levels of success in mate choice tests over the whole season. (See text for details).

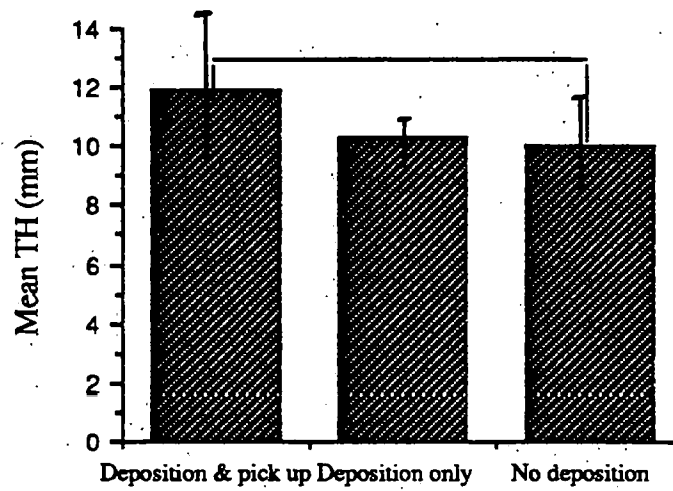


Fig. 6.3 Mean tail heights for males that achieved different levels of success in mate choice tests for 'early' in the season. (See text for details).

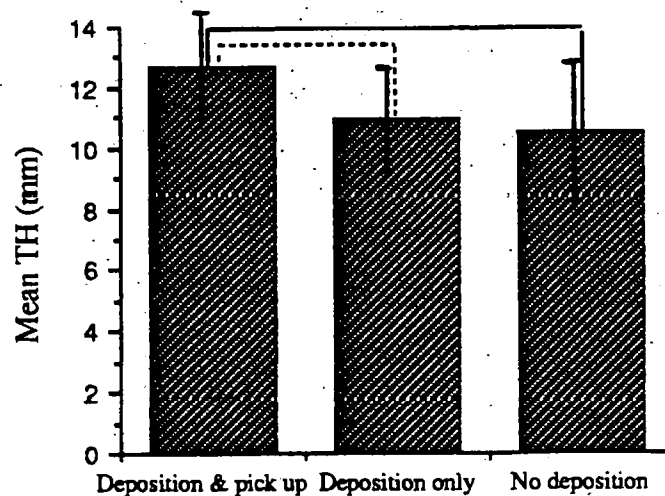


Fig. 6.4 Mean tail heights for males that achieved different levels of success in mate choice tests for 'later' in the season. (See text for details).

Fig 6.3 shows the data for the early part of the season, when the females are highly responsive. At this stage in the season the males that had their sperm picked up had significantly higher tails (mean=11.94mm;n=16) than males that did not achieve deposition (mean=10.04mm;n=25) (see first and third columns in Fig 6.3). However, there was no significant difference (t-test; $p=0.19$) between the tail heights of successful inseminators (mean=11.94mm;n=16) and those males that deposited spermatophores but did not achieve insemination (mean=10.33mm;n=6).

In contrast, later in the season (Fig 6.4), successful males had significantly deeper tails (mean=12.7mm;n=13) than both those males that did not deposit (mean=10.5mm;n=153) (t-test; $p=0.001$) and those males that achieved deposition but not insemination (mean=10.92mm;n=12) (t-test; $p<0.03$).

These results indicate a number of points. In the early part of the season females were not actually as indiscriminate as they appeared (see section 4.4.1). Therefore, even though males may not have developed their crests maximally, there is still some discrimination for deeper tails at this early stage of the season. However, it is interesting to note that females appear to be even more discriminating later in the season: males had significantly larger tails at both the deposition and the pick up stages. This is, as has already been noted, the stage in the season when the males have developed their crests maximally. It is also noteworthy here that the THs of the two sets of experimental males (early [mean=10.72mm;n=42] and later [mean=10.69mm;n=173]) were compared (t-test) and were found not to be significantly different ($p>0.1$). This suggests that actual discrimination was observed and the result was not just because males have larger tails later in the season.

The result from the first two aims of this chapter have been described. They are discussed fully in Section 6.5 in relation to the results of the other experiments.

6.4.3 Spermatophore Pick Up

This section discusses the results of aim number 3. It has already been reported (Section 6.4.1) that the mate choice experiment carried for aims 1 and 2 of

this chapter resulted in very few inseminations. General information on these depositions was given at the beginning of Section 6.4.1. Three encounters had one deposition, four resulted in two depositions, four in three and a single encounter had four depositions. Clearly these data are too few to examine any patterns in pick up.

The motivation experiment (Chapters 4 and 5) data were also studied. In a similar fashion to the previous section (6.4.2) the data for all the encounters were examined together first, then broken down into early and later parts of the season.

The table below (Table 6.1) firstly summarises the details of the spermatophores deposited.

	OVERALL	EARLY	LATER
Total no. encounters	242	48	194
No. with deposition	47 (19%)	23 (48%)	24 (12%)
% pick up (of total)	12%	33%	7%
% pick up (of dpstns)	63%	70%	54%

Table 6.1 Details of spermatophores deposited in motivation experiment (see text for details).

A number of general points emerge from these data, especially when the early and later stages are compared. Over both parts of the season roughly the same number of encounters with deposition occurred (23 early, 24 later) but as percentages of the total number of encounters, they are very different (48% early, 12% later). These results show that around half of all inseminations over the season occur over a short period at the beginning (this has already been discussed in a different context in Section 4.4.1). However, the final row of Table 6.1 suggests that when spermatophores have been deposited insemination may be more likely early in the season than later (70% successful early in season, 54% later).

It is also interesting that, for the later part of the season (see Table 6.1), the percentages of encounters where deposition occurred (12%) and where insemination

resulted (7%) are very similar to those obtained in the mate choice experiment (11% and 8%). This may suggest that this rate of insemination is typical for courtships that occur after the beginning of the breeding season.

The actual pattern of spermatophore pick up will now be discussed and compared with Halliday's (1983a) data (reproduced in Fig 6.5). Fig 6.6 shows all the data for the motivation experiment. What is immediately striking is how different these data are from those in Fig 6.5. The pattern of pick up observed here is almost a reversal of that found by Halliday (1983a); there is a definite trend for earlier spermatophores in a sequence to be picked up rather than later ones.

When the data are divided into early and later stages (Figs 6.7 and 6.8) the tendency for the earlier spermatophores to be picked up is even more evident in the early part of the season. However, the tendency is still apparent later in the season (Fig 6.8). Thus, these data provide no support for Halliday's (1983a) findings and, in fact, suggest a reverse situation in this study: earlier spermatophores in a sequence are favoured by females. The significance of these results are discussed later (Section 6.5).

6.4.4 Familiar and Unfamiliar Males

This part of the work was carried out in 1990 on unmated females (aim 4, Section 6.2). Although a number of females failed to respond (perhaps because time constraints meant that the experiments were carried out rather late in the season) some interesting results emerged. Table 6.2 shows the results from the 38 females tested with males on two consecutive days.

	1st DAY	2nd DAY
SAME MALE (N=19)	8/19	3/19 (no reinseminations)
DIFFERENT MALE (N=19)	7/19	6/19 (2/6 reinseminations)

Table 6.2 Results of presenting 38 females with the same male or a different male on successive days

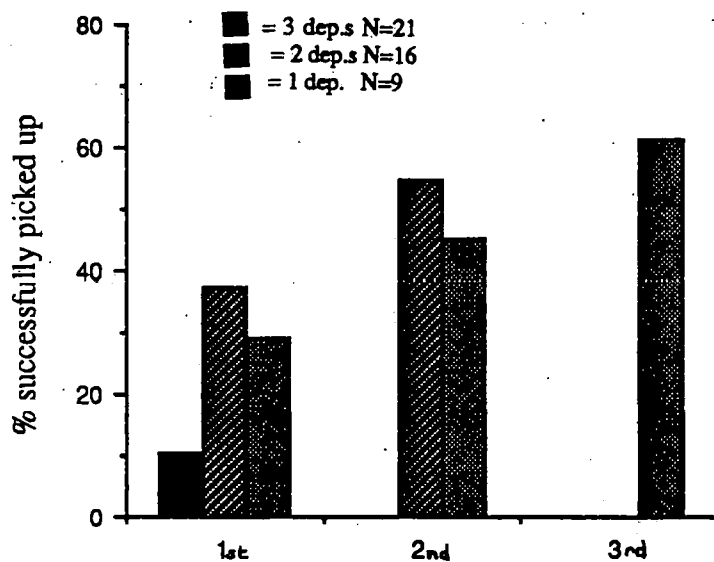


Fig. 6.5 The proportion of spermatophores picked up in relation to their order within a courtship encounter and to the total number deposited during an encounter. From Halliday (1983a).

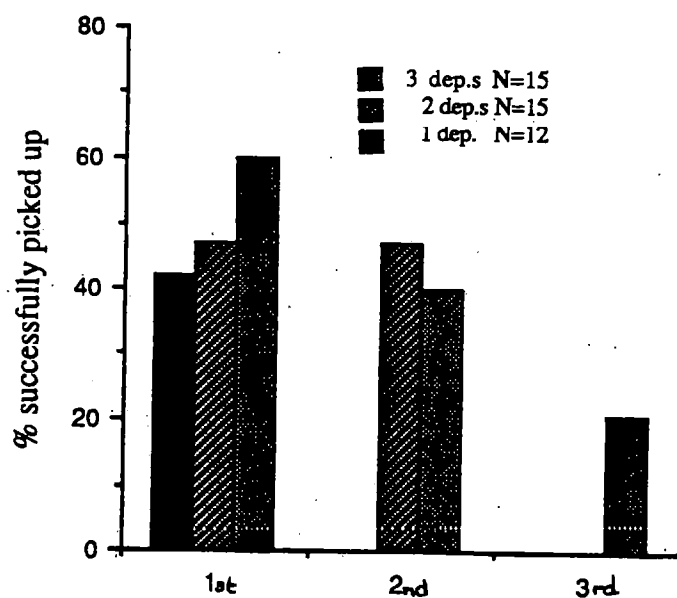


Fig. 6.6 The proportion of spermatophores picked up in relation to their order within a courtship encounter and to the total number deposited during an encounter. Data for whole season.

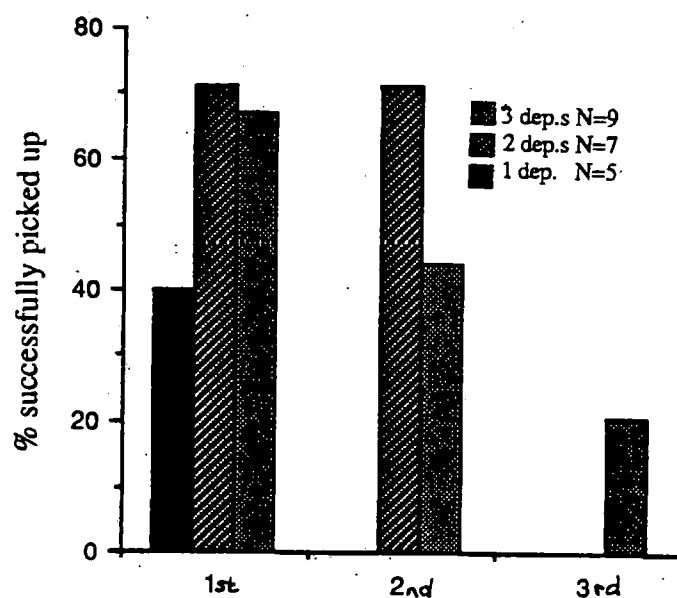


Fig. 6.7 The proportion of spermatophores picked up in relation to their order within a courtship encounter and to the total number deposited during an encounter. Data for 'early' in season.

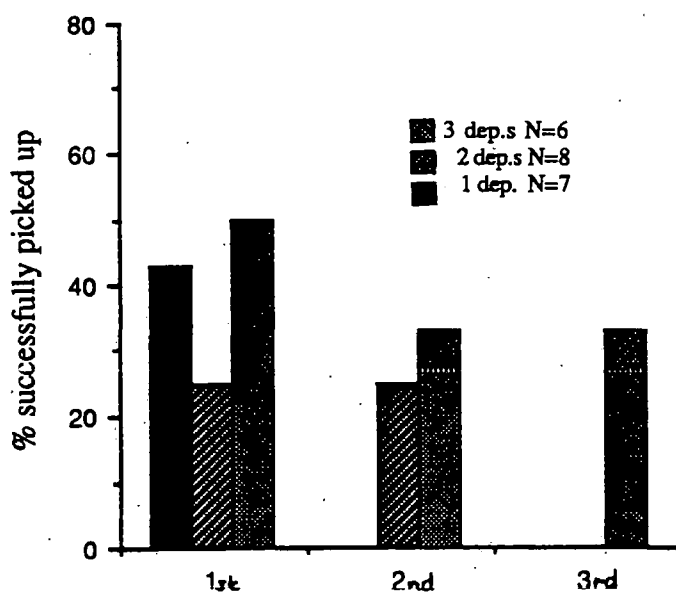


Fig. 6.8 The proportion of spermatophores picked up in relation to their order within a courtship encounter and to the total number deposited during an encounter. Data for 'later' in season.

The results (though non-significant with a χ^2 test; $p>0.1$) indicate trends that are worth further investigation. Females appear to have been inseminated at about the same rate when they were presented with a different male each time (i.e. all values in the table except the top right). When presented with the same male again only three of the females became inseminated. Furthermore, on the second day with a different male, 2/6 of the inseminations were individual females becoming inseminated for the second time. No repeat inseminations occurred when the same males were presented. Though the data here are few, they suggest that females may be 'aware' that a male is familiar to her and be less likely to mate with him. A recent laboratory study with *Desmognathus ochrophaeus* by Donovan & Verrell (1991) has found that courtship trials between familiar pairs are significantly less successful than those between unfamiliar pairs. This result lends some weight to the tentative results found here and suggests that this phenomenon is worth further investigation.

6.4.5 Choice of Two Males

16 females were tested in this experiment but only four of them responded to the test situation (see Table 6.3). Again these unmated females had to be left until quite late in the season (mid May) before they could be tested which may have contributed to the lack of response by the females.

Large-crested male (TH)	S'phores Picked Up	Small-crested male (TH)	S'phores Picked Up
9 mm	0/2	8 mm	2/3
13	2/4	8	0/1
14	1/1	11	0/1
11	0/2	6	0 deposited

Table 6.3 Spermatophores deposited and picked up by females in a choice situation. TH=tail height.

If the females responded at all they usually courted both males at the same time and the courtship progressed rapidly. In contrast with Verrell (1983) the females did not seem to find the situation aversive but appeared rather to take advantage of it. In the small number of encounters observed, the females approached the males so rapidly that both males had a chance to court. In 3 of the 4 cases where deposition occurred, females elicited spermatophores from both of the males, although the data are too few to be able to determine whether females were discriminating between males in any way.

When courtship progresses so quickly two males might actually benefit from courting together rather than the situation being 'interference' (see Verrell 1984a). This way one male could 'rest' after deposition and allow the other to court the female, thus maintaining her attention. The females may benefit from eliciting spermatophores from more than one male at once and thus be in a position to exercise a direct choice between the two males. This work is certainly worth further investigation.

6.5 Discussion

No evidence was found in any part of this work for size-assortative mating. Recently Verrell (1991) has reported a size-dependent preference in *T. vulgaris*, using proximity as an indicator of choice. He suggests that mating with an individual of approximately the same size may be adaptive since it could increase the chances of sperm being picked up. However, perhaps proximity indicates one stage in the process of choice making which is assortative, but using insemination as a rather more critical indication of choice does not seem to reflect this relationship.

When THs of males are considered, females do discriminate for larger tails, even early in the season when the secondary sexual characters are less well developed. Green (1991) also reports a female preference for higher tails at the spermatophore pick up stage. However, females in this study also appear to become more discriminating later in the breeding season. Females mate more readily early in the

season but later perhaps require more courtship from the male; far fewer encounters end in insemination.

However, from what has been discovered about female sexual responsiveness in Chapter 5, it may be less important that males develop their crests maximally after the beginning of the season. If, as Baker (1992) suggested for *T. cristatus* crest height is related to foraging in the terrestrial phase (body condition on arrival at the pond), perhaps males develop their crests initially using these resources. Later, more recent foraging in the present season (Green 1991) may become important in developing a larger crest and maintaining it. Crest height could then be an indicator of foraging ability on two counts. Thus it appears that females are able to and do discriminate larger crests, and furthermore, it may be adaptive for them to do so.

In this work, earlier rather than later spermatophores in a sequence were found to be favoured by females, especially early in the season. This disagrees with Halliday's (1983a) data and its consequent interpretation as a subtle form of female choice. Green (1991) states that his results also do not agree with those of Halliday (1983a) but he does not elaborate further. Halliday's data were pooled from many experiments (pers. comm.) which may have confused different patterns of pick up if there are any seasonal effects involved.

Pick up of earlier spermatophores in a sequence could actually be adaptive for the female. Early in the season it would leave more time to court one male and then go onto other males and multiply mate. Also, Waights (pers. comm.) has found a slight trend for earlier spermatophores in a sequence to contain more sperm than later ones. Again it would seem then possibly advantageous to pick up earlier spermatophores.

The smaller experiments carried out in 1990 have already been discussed fully (Sections 6.4.4 and 6.4.5). These both require further study, especially as so many of the season's inseminations occur over such a short period and the females seem much less averse to approaches by males at this stage. It can be normal in a natural situation for groups of males and females to occur together (per. obs.) and may

have advantages for both sexes, especially if the females can indeed discriminate between familiar and unfamiliar males.

As has been suggested previously, the natural situation needs considerable further investigation. Until much more is known about this area, it is difficult to draw conclusions about the existence of any sampling strategies (e.g. see Janetos (1980)) in female smooth newts. Recent work by Bakker & Milinski (1991) examines sequential female choice in the stickleback *Gasterosteus aculeatus* L. This approach could be of value in elucidating the situation for smooth newts, since they are probably subject to simultaneous and sequential choice situations, perhaps at different times over the season.

What emerges from all the mate choice studies presented here is the lack of knowledge about female perception of the male and his display. The mechanisms by which these 'choices' that the females appear to make are not known. It may be, for example, that larger-crested males simply have a more effective display than a small crested one and are thus more likely to achieve insemination. The females would need only to be selectively responsive, and not actively able to discriminate between males.

For a full understanding of mate choice the study of how each sex perceives the other is vital. The next chapter goes on to examine in detail the female's perception of the male and the relative importance of aspects of his morphology and display.

The previous chapter showed how female sexual responsiveness varied over the season. Male display was found to be more important to the female's sexual motivation later in the season, which may reflect the results found here. The results of these two chapters and the following one are drawn together in Chapter 8.

CHAPTER 7

The Importance to the Female of Olfactory and Visual Stimuli in Courtship

7.1 Introduction

Communication is at the heart of courtship. Each of the participating individuals sends and receives signals but, to human perception at least, the signals sent out by the male are usually the more conspicuous. This is especially obvious for species where the male performs an elaborate courtship display; newts are a good example. The female responds to the male's courtship but it is difficult for a human observer to say exactly to which cues in the male's courtship she may respond. Firstly, a human may not perceive the courtship in the same way as the female newt does and secondly, some cues may be more important than others and their importance may change at different stages in the courtship, something human perception may not be able to detect.

There is likely to be variation between individual males in the signals they send to a female. Similarly, she may respond differentially to signals from different males, or, differently to those of the same male at different times, according to, for example, her sexual motivation. These variations provide the basis of a mechanism for female choice (see Section 6.1 for definition) and understanding the mechanisms underlying this communication during courtship may illuminate how sexual selection by female choice could operate (Ryan 1990).

This part of the project examined the study of female choice from a perspective different from that taken in the rest of the study. A more experimental approach was adopted and specific hypotheses were tested. Instead of putting individuals together and obtaining information about the outcome of a particular pairing, the specific cues that a female might receive from the male during courtship were examined. If the response of the female to particular cues and combinations of cues could be determined, their importance in mate choice might be indicated.

The male newt's secondary sexual characters are to human perception obvious candidates for sexual selection through female choice (see Chapter 6). However, it is not known how the female really perceives them. Tinbergen & Ter Pelkwijk (1938) identified the importance of odour in courtship and suggested that colour, movement and the shape of the male may also be important to the behaviour of the female. Tinbergen (1953), and later Halliday (1974), described the male's courtship as incorporating visual, olfactory and tactile stimuli and assigned different aspects of the display to these three categories of stimuli. These distinctions between the different aspects of the courtship and their functions as stimuli for the female have become accepted in the literature. Tinbergen (1953) himself says that experimental study is necessary to test the 'obvious' suggestion that different parts of the courtship provide different stimuli. Perhaps it is because it seems so obvious that it has never really been tested.

However, since these untested observations have become an important part of discussions about the extent of sexual dimorphism and about phylogeny in *Triturus* spp. (e.g. Malacarne & Giacoma 1986; Giacoma & Sparreboom 1987; Giacoma & Balletto 1988), it would be very interesting to know if these supposed sets of stimuli are actually perceived as such by the female, and how important they are in altering her behaviour. Human observers may see a large crest and assume the female does also, but it may be, for example, that a large crest enables the male to better direct tactile or olfactory stimuli to the female rather than being a predominantly visual stimulus (as previously suggested by Halliday 1977b; Raxworthy 1989).

This experiment was therefore devised to investigate the effect and importance of two of the different types of stimuli presented to the female by the male during courtship: olfactory and visual. The stimuli, or cues, are described as being of two types because two separate sensory systems are assumed to be stimulated in the female. The sources of the two sets of cues were briefly as follows (details are given in Section 7.2).

The olfactory cues were obtained by eliciting static display from males before the experiment took place and in the absence of the test female. The cues consisted of the putative courtship pheromone that the male produces during static display (Halliday 1974; Malacarne et al 1984).

The visual cues that the female received in the experiment consisted of a live male newt, anaesthetised and placed in a modified strait-jacket (see Halliday 1975). This enabled all the visual characteristics of a real male to be presented to the female, whilst preventing any courtship display occurring.

While this method of presenting visual stimuli means that no courtship pheromone (i.e. for this experiment this means that which is actually produced during male display) reaches the female, it is likely that she may receive certain olfactory cues about a male if she is near enough to him to simply smell his presence. It is evident from personal observation that males and females in close contact appear to sniff each other, either with the snout in contact with the other individual or with a small distance between them. This might seem a problem because it means that not all olfactory cues would be eliminated when it was intended that only visual cues were to be presented to the female. However, it actually provides a potentially useful source of information about female behaviour, as her behaviour may change when in close proximity of the male. The experiment sought to take account of this and any biases that could have occurred in the results because of it (see Section 7.3).

7.2 Aims

The primary aim of the experiment was to determine the effect on the female and the importance to her of the olfactory and visual courtship cues delivered by the male. By presenting olfactory and visual cues to females separately, and in combination, it was hoped that their relative importance in female choice might be determined.

More specifically, the behaviour of the female was to be compared, in detail, in the presence and absence of pheromone (with no visual stimulus present). This was

to see if the presence of courtship pheromone had any effect on the female's behaviour, bearing in mind that, at this stage, she had received no courtship from the male, and no visual stimuli (in the form of the male himself) were present.

The second part of the experiment sought to investigate whether the female would make any discrimination between the visual stimulus of a large-crested versus a small-crested male. This was investigated in the absence of olfactory cues (associated with courtship pheromone) and also with prior and continued exposure to pheromone.

7.3 Materials and Methods

7.3.1 Animals

The animals were collected as they migrated to Great Linford pond, as explained in Chapter 2. Over the nights of 19/20 February 1989, 42 females were collected. This was the full number needed for the experiment and they were collected over two consecutive nights, which would ensure that the effects of time of season (if any) were kept to a minimum (see Chapter 4). Many fewer males were caught on these nights.

After each night of collecting the animals were put in small plastic boxes lined with damp tissue paper and with damp paper refugia. These were kept in the fridge (at 4°C) until enough male newts had been collected to complete number needed for the experiment. This method of keeping the terrestrial newts has been used successfully and with no apparent harmful effects (J.Baker, pers. comm). Just after these first newts were collected the weather became very cold and dry (at the end of February and into the beginning of March) with the result that no further newts migrated during this time.

Because the males develop their secondary sexual characteristics only after entering the water, they were put into their migration tanks (see Section 2.2.3) before the females were removed from the fridge and put into theirs. However, by 2nd March the males had not moved from under the stones in the dry end of the migration tanks, and were still terrestrial. All the females were put into migration tanks on 2nd March

but no newts migrated into the water until later in March when the weather became much warmer. The females were given about a week to adjust to being in water and then the experiments could begin.

The problem of having males with only poorly developed crests remained. Males with large crests were required for anaesthetising and using as a visual stimulus in the experiment. In the past, attempting to catch newts actually in the water at Great Linford had proved very problematic as the newts are seen only rarely (see Section 2.1). However, an attempt was made to obtain some as it was considered important that the male newts came from the same pond as the females. (There is some evidence that females prefer to mate with males from other ponds [Green 1989]; this could potentially have had a confounding effect upon the results of this experiment.) Six hours in the pond with two people netting finally yielded five males, of which three had well developed crests and could be used to start the experiment.

Tests were carried out between 28/3/90 and 5/4/90. They were completed over as short a period of time as was possible to eliminate any effect of time of season.

7.3.2 The Observation Tank

The observation tank (20x25x40 cm) was set up as shown in Fig. 7.1. A grid marked in square centimetres was placed under the tank so that it was possible to determine distances between the female and the male and also to divide the floor into four Sections. These were designated 'very near', 'near', 'far' and 'very far', according to the position of the male.

The tank contained a standard volume of 5000ml aged tap water for each test and the water was changed for each test. When pheromone was to be used, a standard volume of water containing the pheromone (800ml, see 7.3.3 below) was added to 4200ml plain water, so that the total volume in the tank was the same for all tests.

7.3.3 The Olfactory Stimulus

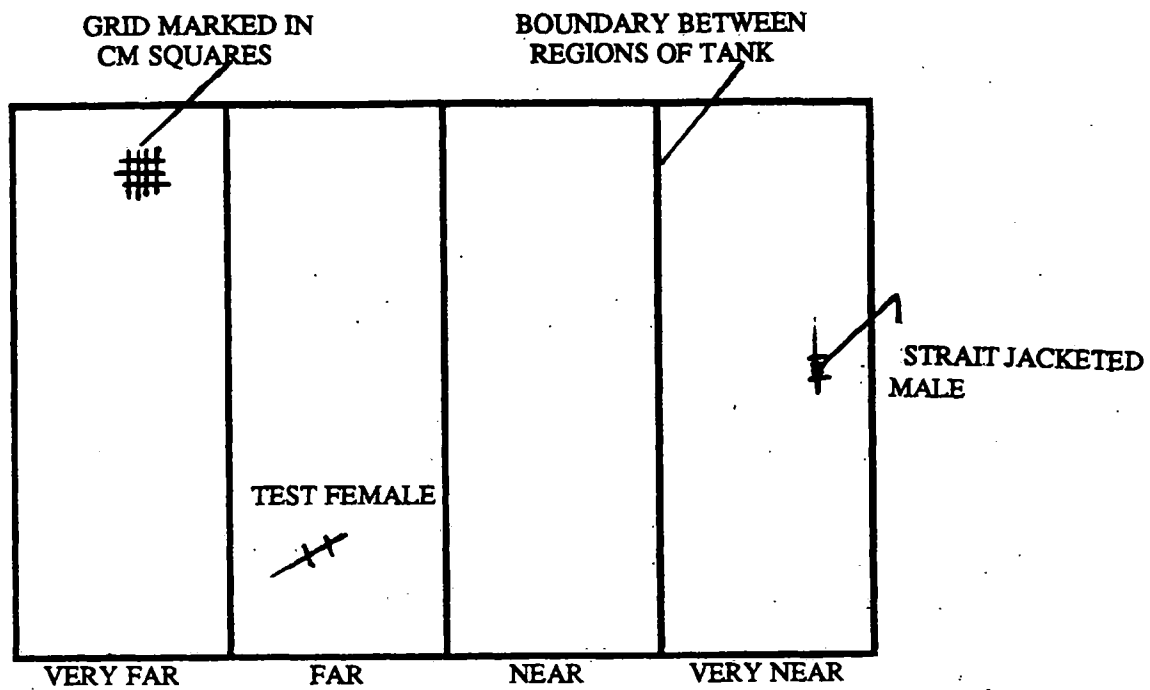


Fig. 7.1 Observation tank set-up, seen from above. (See text for details.)

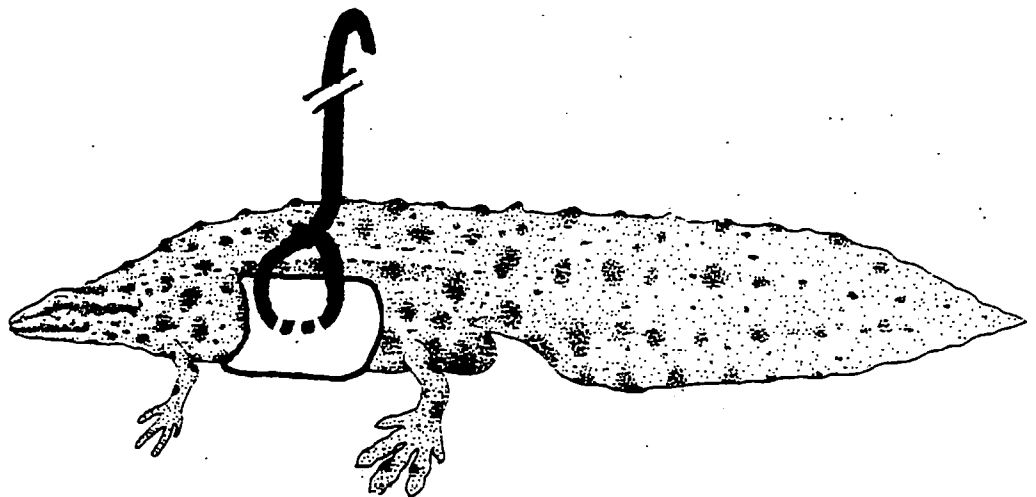


Fig. 7.2 Male newt positioned in a strait-jacket. (See text for details.)

The large and the small crested males that were to be used as visual stimuli were placed in a small tank (30x15x10 cm) containing 3200ml aged tap water. By using a strait-jacketed female to approach the males (see Halliday 1975) they were stimulated to perform static display. Ideally, the same amount of display from each of the two males was required, but it was almost impossible to obtain this. At least ten whips, sixty seconds of fan and ten seconds of wave were aimed for from each of the males. Usually some display was obtained from both males but if one male was reluctant, a proportionately greater amount of display was elicited from the other. On a very few occasions if no display whatsoever could be obtained from one of them (this was occasionally the case if the ambient temperature was very low), presence in the water had to suffice as the olfactory contribution from that male.

Obviously this situation was not ideal but the olfactory stimulus was as standardised as possible under the circumstances. In each case the pheromone was elicited from the two males with which the female would later be presented, so the smell of the males would 'match' the visual characteristics that the female saw. It was not known if this was important for the female but it seemed a good idea to take it into consideration. The pheromone was elicited in the same amount of water in each observation period (3200ml). This water was stirred to distribute the pheromone evenly. 800ml was taken out for each of the tests, when required, and added to the 4200ml of plain water already placed in the observation tank. Although the amount of pheromone elicited will not have been exactly the same in each case, at least the volume of water in which it was distributed was standardised.

7.3.4 The Visual Stimulus

Two males, one large- and one small-crested, were used for the whole of one observation session. They were anaesthetised in MS 222 (1:1000, Sandoz) to render them temporarily immobile. A strait-jacket was made along similar lines to that developed by Halliday (1975). However, certain modifications were necessary to

accommodate the male's crest and allow it to stand up from the dorsal surface in a normal fashion (Fig 7.2).

A 15mm length of flexible clear plastic tubing of 10mm bore was cut and a slit made along its length. The corners were rounded so as not to cut into the newt's body. Half a wire coathanger was fixed through the tubing to provide a means for manipulating the newt placed inside the tubing (see Fig 7.2).

During the visual stimulus presentation period (see Section 7.3.5), the male was held at the chosen end of the tank (see Fig. 7.1). The male was moved by flicking the wrist very lightly so that the tail flexed a little. This was done approximately every fifteen seconds during the six minutes. Thus the live male provides all the visual characteristics of a real animal and, in addition, the visual stimulus of movement.

7.3.5 General Routine of Tests

This section and Fig 7.3 give an overview of how the experiments were performed. As well as investigating the general aims already mentioned, this experiment also provided the opportunity to investigate some of the behavioural actions that females performed at certain times, in a more quantitative fashion. Details of these and the information recorded are given in Section 7.4.

A total of 38 females were tested, half of which were exposed to pheromone before being presented with the visual stimuli (large- and small-crested males), the other half were not exposed to pheromone beforehand.

The female was introduced into the observation tank and allowed six minutes to settle (the olfactory stimulus presentation period). Certain behavioural actions were noted (see Section 7.4.2) during the third and the sixth minute of this time. This procedure was followed both for the females that were exposed to pheromone in the observation tank and those which simply had plain water in the tank. This allowed the behaviour of the females in the presence of the pheromone (but in the absence of any visual stimulus) to be compared with that of those females with no exposure to pheromone.

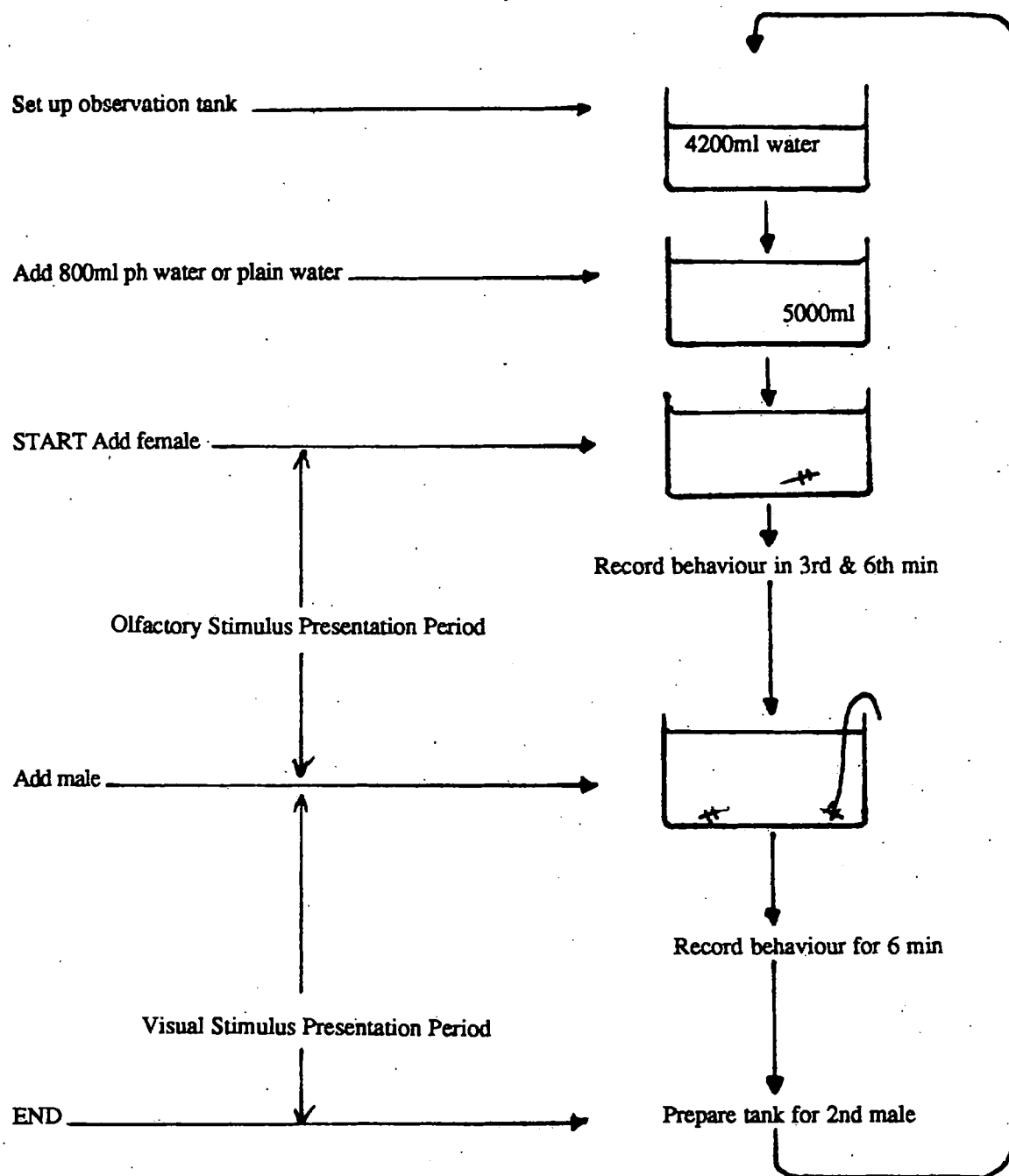


Fig 7.3 Overview of experimental protocol. Ph = pheromone. (See text for details.)

At the end of the olfactory stimulus presentation period, the first of the two males was presented to the female. The next six minutes made up the visual stimulus presentation period. During this period various parameters and female behavioural actions were noted as explained in Section 7.4.

At the end of this test, the female was removed briefly from the tank while the water was changed for the second test, in which the second male was presented. The order of presentation of the large- and small-crested males was decided by tossing a coin. Similarly, the end of the tank into which the male was to be placed was decided by the tossing of a coin. The two males were not presented at the same time because it would not have been possible to determine whether the position of the female was an indication of her actual preference for proximity to one male, or her avoidance of the other. The sequential presentation of the males, however, introduced the potential problem of order effects. The order of presentation of the males was randomised to counteract this but was also noted so that order effects could be examined in the data.

The female was then put back into the observation tank and allowed to settle for six minutes as before, her behaviour being noted in the third and sixth minute (olfactory stimulus presentation period). Following the same procedure as in the first test, the second male was then presented to the female at the end of the sixth minute and her behaviour recorded as before (visual stimulus presentation period).

Four females were tested in one observation session (a morning or an evening), two with prior exposure to pheromone, two without. The olfactory stimulus (pheromone in water) was obtained from the stimulus males to test two females twice (once with each of the two males). These four tests were done first (as it was thought the pheromone might degrade with time), then two more females were tested with the two males, but this time without prior exposure to pheromone (four more tests).

To summarise (see Fig 7.3), half the females ($n=19$) were exposed to pheromone in their test water (for both the males) and half had no exposure to pheromone. Then each female ($n=38$) was tested with the two different visual stimuli

(large- and small-crested males) to see if there was any difference in female behaviour when presented with a large-, compared with a small-crested male. The possibility that prior and continued exposure to pheromone during the test would make a difference to the behaviour of the females when presented with the large- and small-crested males was also investigated (i.e. whether there is any interaction of olfactory and visual stimuli).

7.4 Behaviour Patterns Recorded and Hypotheses Tested

7.4.1 Scores Recorded in Tests

The female behaviour patterns scored were those that have been previously observed and were apparently associated with sexual responsiveness. This was the general rationale for the design of the experiments. To avoid unnecessary repetition the specific rationale for each hypothesis is discussed with the result of the statistical test of each hypothesis (Section 7.5). The scores recorded for each female tested were as follows:

- 1) *Number of sniffs* - the number of flexions of the buccal surface observed.
- 2) *Number of head movements* - any movement of the head while the female was stationary.
- 3) *Number of open-mouth sniffs* - a large opening of the mouth, something like a yawn, where water is drawn into the mouth.
- 4) *Number of boundaries crossed* - i.e. boundaries between the different regions of the observation tank. This was used as an index of the female's level of activity.
- 5) *Time spent very near, near, far and very far from the male* - i.e. in the different regions of the observation tank.
- 6) *Time the female spent within ten cm of the male*. Ten cm is approximately the body length of a newt and during courtship the two individuals are rarely more than ten cm away from each other.
- 7) *Rate of sniffing when within ten cm and when not within ten cm*. This

was calculated from 1) and 6) above.

8) *Any other behaviour patterns* - any potentially interesting behaviour patterns, not recorded as above, were noted.

All the scores (except 5-7) were recorded in the third and sixth minutes of the olfactory stimulus presentation period. In the visual stimulus presentation period, all the scores were recorded, as totals for the whole six minutes.

7.4.2 Hypotheses Tested in Olfactory Stimulus Presentation Period

It is important to note that, in this chapter, the hypotheses are not simply general ideas about some aspect of an animal's behaviour. The experiments were carefully designed, including the statistical tests to be used for analysis. In this way, *specific* hypotheses were generated from the experimental design and the planned analyses. It is these specific hypotheses that are shown in this and the following section.

- a) Females sniff more when pheromone is present than when it is absent (this hypothesis was tested for the third minute and for the sixth minute).
- b) Females sniff more at the beginning of the olfactory stimulus presentation period (during the third minute) than at the end (during the sixth minute) (this hypothesis was tested both in the presence and in the absence of pheromone).
- c) Females perform more head movements when pheromone is present than when it is absent.
- d) Females perform more open-mouth sniffs when pheromone is present than when it is absent.
- e) Females cross fewer boundaries (i.e. show less activity) when pheromone is present than when it is absent.

7.4.3 Hypotheses Tested in Visual Stimulus Presentation Period

Each of the following hypotheses were tested more than once, under different conditions, according to the symbols: * ^ ~. The * indicates that the hypothesis was

tested for females both in the presence of pheromone and in the absence of pheromone; the ^ indicates that the hypothesis was tested for the large- and the small-crested male; the -- indicates that the hypothesis was tested when within ten cm and not within ten cm of the male. These are explained in more detail in the results, Section 7.5. Order effects were also investigated as explained in Section 7.5.

- *a) Females sniff more with a large-crested than with a small-crested male.
- ^b) Females sniff more with pheromone present than when it is absent.
- *c) Females perform more head movements with a large-crested than with a small-crested male.
- ^d) Females perform more head movements when pheromone is present than when it is absent.
- *e) Females perform more open-mouth sniffs with a large-crested than with a small-crested male.
- ^f) Females perform more open-mouth sniffs when pheromone is present than when it is absent.
- *g) Females cross fewer boundaries with a large-crested than with a small-crested male.
- ^h) Females cross fewer boundaries when pheromone is present than when it is absent.
- *i) Females spend more time near to a large-crested than to a small-crested male.
- ^j) Females spend more time near to the male with pheromone present than when it is absent.
- *k) Females spend more time within ten cm of a large-crested than a small-crested male.
- ^l) Females spend more time within ten cm of a male with pheromone present than when absent.
- *~m) Females sniff faster with a large-crested than a small-crested male.
- ^~n) Females sniff faster when pheromone is present than when it is absent.
- *^o) Females sniff faster when within ten cm of a male than not within ten cm of him.

7.5 Results

7.5.1 The Stimulus Males

The stimulus males were chosen to be of roughly the same body length but to differ markedly in crest height. Thus they differed most in the development of their most obvious secondary sexual character. The two males used in each test period differed in SVL by less than 2mm; their tail heights differed by at least 2mm.

In all the sections that follow, the results of the hypotheses tested are presented in a table. This may seem a little unwieldy but it is intended to enable quick reference to be made to all the specific results of the statistical tests performed. Thus all the results are included for the sake of clarity, if somewhat to the detriment of brevity.

7.5.2 The olfactory stimulus presentation period - *number of sniffs*

The results of the tests of hypotheses a) and b) (Section 7.4.2) are shown below in Table 7.1 and in Fig 7.4.

1.a) Females sniff more when ph is present than when it is absent (3rd min)	(t-test) NS
2.a).7 Females sniff more when ph is present than when it is absent (6th min)	(t-test) $p < 0.05$
3.b) Females sniff more during the 3rd minute than during the 6th minute (+ph)	(Wlcn) NS
4.b) Females sniff more during the 3rd minute than during the 6th minute (-ph)	(Wlcn) $p < 0.01$

Table 7.1 Hypotheses tested and results for no. sniffs performed by females in olfactory stimulus presentation period (1st test). Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

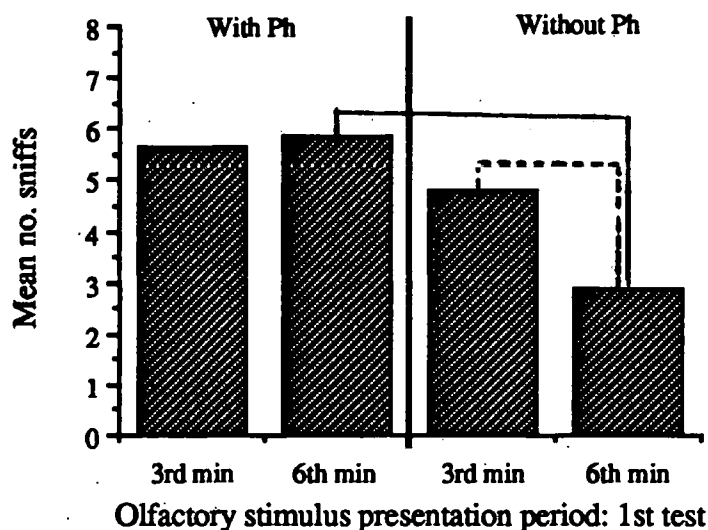


Fig 7.4 Number of sniffs performed by females in olfactory stimulus presentation period (1st test). Ph = pheromone; — = $p < 0.05$; = $p < 0.01$. (For with ph, $N=18$; for without ph, $N=16$.)

The distinction between the third and the sixth minute was used as it was thought that the female may sniff at a high rate in the third minute whether pheromone was present or not, simply because the tank water would constitute a novel environment. Indeed, females sniffed more in the presence of pheromone than in its absence in the sixth minute (2, Table 7.1; Fig 7.4), but not in the third minute (1, Table 7.1; Fig 7.4). Also, when pheromone was absent females sniffed more in the third minute than in the sixth (4, Table 7.1; Fig 7.4), but not if pheromone was present; i.e. the number of sniffs was high in the third minute and remained high in the sixth minute.

These results suggest that females did indeed sniff at a high rate when first encountering a novel environment, regardless of whether pheromone was present or not. However, when pheromone was present, they *continued* to sniff at a high rate. This is also reflected in that they sniffed significantly more in the sixth minute of the test when pheromone was present than when it was absent (2, Table 7.1).

Although data were collected in the olfactory stimulus presentation period for each of the two tests (two males) in succession, the results above are from the first test

only. It was thought that the pheromone might have a lasting effect which may confuse the issue. (Although this criticism could also be levelled at the visual stimulus presentation period data, the situation for analysis is different as the two conditions {large and small crest} both had to be tested; the order effects for these experiments are investigated and discussed at the appropriate point below.) The data for the second olfactory stimulus presentation period are shown below.

- | | |
|--|---------------------|
| 1.a) Females sniff more when ph is present than when it is absent (3rd min) | (t-test) $p < 0.05$ |
| 2.a) Females sniff more when ph is present than when it is absent (6th min) | (t-test) NS |
| 3.b) Females sniff more during the 3rd minute than during the 6th minute (+ph) | (Wlcn) NS |
| 4.b) Females sniff more during the 3rd minute than during the 6th minute (-ph) | (Wlcn) NS |

Table 7.2 Hypotheses tested and results for number of sniffs performed by females in olfactory stimulus presentation period (2nd test). Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

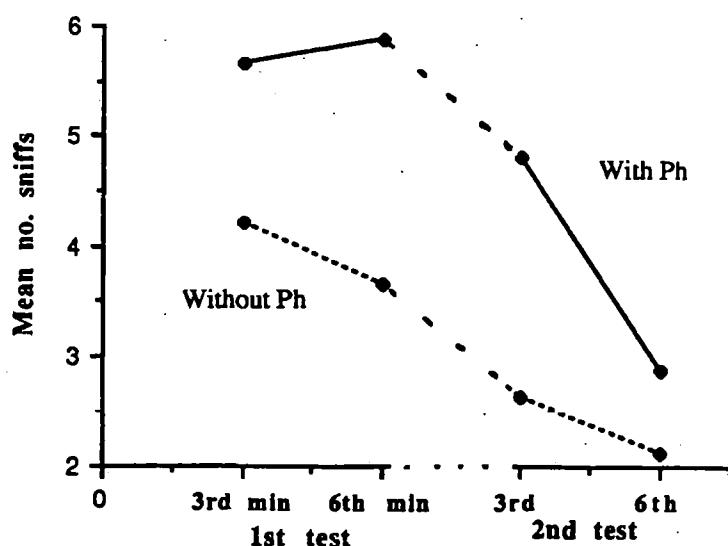


Fig 7.5 Patterns of no. sniffs performed by female in 1st and 2nd olfactory stimulus presentation periods. (See text for details).

The pheromone did appear to have some kind of lasting effect since the pattern of results was not the same as that found for the first olfactory stimulus presentation period. In the third minute of this second test, females did sniff more when pheromone was present than when it was absent (1, Table 7.2), but in the sixth minute they did not (2, Table 7.2). Also in this second test, both in the presence and in the absence of pheromone, females did not sniff more in the third minute than in the sixth minute (3, 4, Table 7.2).

These results indicate that the females did not appear to show the same initial response, in the first and second tests, when first encountering the water in the observation tank. Although the water was changed between the two tests, all the water used came from the same stock so it may not appear as novel to the female the second time around. Similarly, the females seemed to become somewhat familiar with the pheromone by the second test. A significant difference in numbers of sniffs between females in the presence of pheromone compared with those not exposed to it was found in the third minute (4, Table 7.2); the females exposed to pheromone seemed to be familiar with it by the sixth minute (2, Table 7.2).

This is further borne out by the investigation of whether females sniff more in the first test than in the second for each of the categories recorded. The results of this are shown in Table 7.3 below.

1.Comparing no. sniffs between 1st & 2nd tests (3rd min.,+ph)	(Wlcn) NS
2.Comparing no. sniffs between 1st & 2nd tests (6th min.,+ph)	(Wlcn) $p < 0.05$
3.Comparing no. sniffs between 1st & 2nd tests (3rd min.,-ph)	(Wlcn) $p < 0.05$
4.Comparing no. sniffs between 1st & 2nd tests (6th min.,-ph)	(Wlcn) NS

Table 7.3 Hypotheses tested and results for no. sniffs performed by females in olfactory stimulus presentation period, compared between 1st and 2nd tests. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

The general impression from looking at Figs 7.4 and 7.5 is that the amount of sniffing was lower in the second test. The first result in Table 7.3 (see also Figs 7.4 and 7.5) indicates that when pheromone was present females did sniff at a high rate at the beginning of the second test, as they had done in the first, but, in the sixth minute, the number of sniffs were lower in the second test than in the first (2, Table 7.3). In the absence of pheromone, the females performed more sniffs in the first test than in the second in the third minute (3, Table 7.3) but not in the sixth (4, Table 7.3).

Thus it seems that, by the second test, the water without pheromone had lost some of its novelty for the females (they perform fewer sniffs (3, Table 7.3)) and they also appeared to show less of a response to the pheromone (2, Table 7.3).

7.5.3 The olfactory stimulus presentation period - *number of head movements*

The number of head movements recorded was rather low, even when the numbers for the third and sixth minutes were combined as a total for the whole olfactory presentation period. As these were too small for any statistical analysis to be performed, simply the number of females performing head movements was examined. The Table below shows these for the presence and absence of pheromone and for the first and second tests.

With Pheromone	Without Pheromone
14/16 (73.7%) - 1st test	8/16 (50.0%) - 1st test
15/17 (88.2%) - 2nd test	7/16 (43.8%) - 2nd test

Table 7.4 No.s of females performing head movements in olfactory stimulus presentation period.

The hypothesis to be tested by this data was 'females perform more head movements when pheromone is present than when it is absent' (see Section 7.4.2). Whilst this actual hypothesis could not be verified, the data do indicate that more females performed head movements in the presence of pheromone than in its absence. If the data from the first and second tests are combined, a χ^2 test shows that

significantly more females performed head movements in the presence of pheromone than in its absence ($p < 0.05$).

7.5.4 The olfactory stimulus presentation period - *number of open-mouth sniffs*

As with the number of head movements, the numbers of these behavioural actions recorded was very low. Consequently, the actual numbers of females performing open-mouth sniffs are shown instead in Table 7.5.

With Pheromone	Without Pheromone
5/19 (26.3%) - 1st test	3/16 (18.8%) - 1st test
7/17 (35.3%) - 2nd test	3/16 (18.8%) - 2nd test

Table 7.5 No.s of females performing open-mouth sniffs in olfactory stimulus presentation period.

The hypothesis to be tested was 'females perform more open-mouth sniffs when pheromone is present than when it is absent'. The data in Table 7.5 can only suggest that a slightly larger number of females performed open-mouth sniffs in the presence of pheromone than in its absence, but this result is not significant (χ^2 test).

7.5.5 The olfactory stimulus presentation period-*number of boundaries crossed*

Once again too few data were collected to enable the hypothesis shown in Section 7.4.2 to be tested. The actual numbers of females that performed the behavioural action are shown in Table 7.6.

With Pheromone	Without Pheromone
7/19 (36.8%) - 1st test	10/16 (62.5%) - 1st test
12/17 (70.6%) - 2nd test	15/16 (93.8%) - 2nd test

Table 7.6 No.s of females crossing boundaries in olfactory stimulus presentation period.

In both the first and the second tests the numbers of females crossing boundaries was greater when pheromone was absent than when it was present (see Table 7.6). The idea behind the hypothesis 'females cross fewer boundaries when pheromone is present than when it is absent' was that previous observation had suggested that females tend to move around less when interested in a male or in mating. These data show that fewer actual females moved around in the presence of pheromone than in its absence, which lends some weight to the original idea, although this result is not significant (χ^2 test). In the second test, in both the presence and the absence of pheromone, more females seemed to cross boundaries than had in the first test (i.e. they seemed more active), but again this is not significant (χ^2 test).

7.5.6 The olfactory stimulus presentation period - *any other behaviour patterns*

The most striking behaviour pattern noted was thought to be some kind of orientation behaviour. The female would stand still for a few seconds in an alert posture (with head up and legs fully stretched, similar to that described for males {Halliday 1974}), then turn abruptly through about 90° and stand alert once more, facing in a different direction. Sometimes the female might turn as many as three or four times in a minute and continue turning for a couple of minutes; at other times she might repeat the behaviour pattern only once or twice.

It was very interesting to note that she could usually be observed to be sniffing when stationary in the alert posture. It seemed as though she was orientating herself towards something by standing and sniffing in one direction, then turning and sniffing in another direction. This behaviour pattern occurred significantly more often (χ^2 test, $p < 0.05$) when pheromone was present in the water (10/19 cases); indeed it occurred very few times (2/16 cases) when no pheromone was present. As the female is surrounded by male courtship pheromone at this time it would seem adaptive to try and orientate towards the source of the pheromone, especially if she is interested in mating.

7.5.7 The visual stimulus presentation period - *number of sniffs*

The hypotheses a) and b) already outlined in Section 7.4.3 were tested and the results are shown in Table 7.7 below.

1.a) Females sniff more with a large-crested than with a small-crested male (+ph)	(Wlcn) NS
2.a) Females sniff more with a large-crested than with a small-crested male (-ph)	(Wlcn) NS
3.b) Females sniff more with pheromone present than when it is absent (Lcr)	(t-test) NS
4.b) Females sniff more with pheromone present than when it is absent (Scr)	(t-test) NS

Table 7.7 Hypotheses tested and results for no. sniffs females performed in visual stimulus presentation period. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

None of the hypotheses were supported by the data. After the result obtained in Section 7.5.2 (i.e. number of sniffs in the first olfactory stimulus presentation period, see Table 7.1), it may seem contrary on first perusal that 3 and/or 4 in Table 7.7 proved non-significant. On the other hand if the results in Table 7.2 (Section 7.5.2) are considered (i.e. number of sniffs in the second olfactory stimulus presentation period), they may shed some light on this result.

It was found that the females seemed to have become somewhat familiar with the pheromone present in the tank by the second of these tests. Since the visual stimulus presentation period followed on from these, it seems that the females no longer showed any response to the presence of pheromone: they did not perform any more sniffs in its presence than they did in its absence. Moreover, 1 and 2 in Table 7.7 indicate that the presence of the visual stimulus of a male, with either a large or a small crest, seemed not to have made any difference to the number of sniffs the females performed.

However, when the data were examined for order effects an interesting result was found. The following Table (Table 7.8) lists the four hypotheses that were tested, with the results of the tests (see also Fig 7.6).

- | | |
|---|-------------------|
| 1.Females sniff more with 1st male presented than with 2nd male (+ph) | (Wlcn) $p < 0.05$ |
| 2.Females sniff more with 1st male presented than with 2nd male (-ph) | (Wlcn) NS |
| 3.Females sniff more with pheromone present than when it is absent (1st male) | (t-test) NS |
| 4.Females sniff more with pheromone present than when it is absent (2nd male) | (t-test) NS |

Table 7.8 Hypotheses tested to investigate order effects in no.s of sniffs females performed in visual stimulus presentation period. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.



Fig 7.6 No. of sniffs performed by females in visual stimulus presentation period. Ph = pheromone; ___ = $p < 0.05$. (For with ph, $N=17$; for without ph, $N=16$.)

The first result in Table 7.8 shows that females sniffed more with the first visual stimulus male presented than with the second (i.e. regardless of the height of the crest), but only when the females had experienced prior and continued exposure to the pheromone in the water, not if they had not (2, Table 7.8). This indicates that it was not simply the presentation of the first visual stimulus male alone (2, Table 7.8), nor the presence of pheromone alone (3, Table 7.8) that resulted in the females performing more sniffs, but the presentation of the first male in the presence of the pheromone (1, Table 7.8).

This result is even more interesting when the results in Table 7.7 are reconsidered. Here the females appeared to show no response (in terms of number of sniffs performed) to presence or absence of pheromone - it was thought they had become sensitised to the pheromone. However, females sniffed more when presented with the first male than with the second when pheromone was present, (different sizes of crest did not elicit a similar response [Table 7.7], as might have been expected).

Thus the initial and continued exposure to pheromone appears to have affected the females' behaviour only when the first visual stimulus male was presented, and this did not occur when females had not been exposed to pheromone. There is then some evidence for the idea that there may be some kind of interaction between the olfactory and visual stimuli presented to the female.

7.5.8 The visual stimulus presentation period - *number of head movements*

The results of testing the hypotheses c) and d) in Section 7.4.3 are shown below (Table 7.9) and in Fig 7.7.

1.c) Females perform more head movements with a LCr than a SCr male (+ph)	(Wlcn) NS
2.c) Females perform more head movements with a LCr than a SCr male (-ph)	(Wlcn) $p < 0.01$
3.d) Females perform more head mvmnts with ph present than when it is absent (LCr)	(t-test) $p < 0.05$
4.d) Females perform more head mvmnts with ph present than when it is absent (SCr)	(t-test) $p < 0.01$

Table 7.9 Hypotheses tested and results for no. of head movements performed in visual stimulus presentation period. Ph = pheromone; LCr = large-crested; SCr = small-crested; mvmnts = movements; Wlcn = Wilcoxon test.

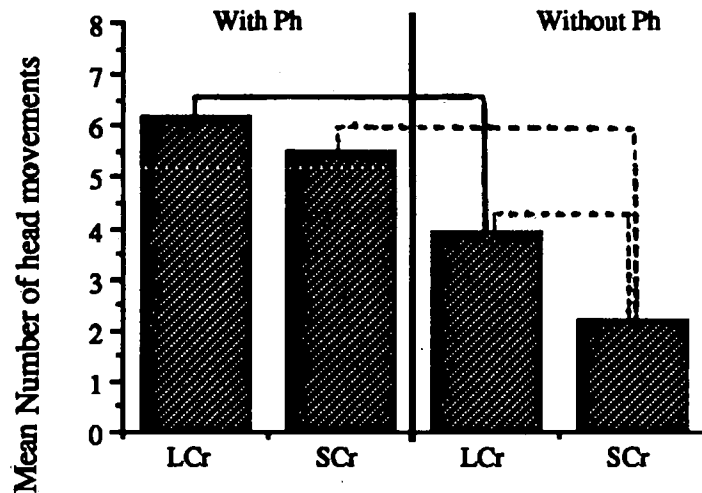


Fig 7.7 No. head movements performed by females in visual stimulus presentation period. Ph = pheromone; LCr = large-crested; SCr = small-crested; __= $p < 0.05$;= $p < 0.01$. (For with ph, N=17; for without ph, N=16.)

Results 3 and 4 in Table 7.9 indicate that the presence of the pheromone had a definite effect upon the number of head movements performed, thus reiterating the results shown for the olfactory stimulus presentation period (Section 7.5.3). For both large- and small-crested males, the number of head movements performed by the females was greater in the presence of pheromone than in its absence (3, 4, Table 7.9; Fig 7.7). Also, when pheromone was present, there was no difference between the number of head movements performed whether the female was presented with a large-crested male or a small-crested male (1, Table 7.9); the number was high in both cases (Fig 7.7). However, when no pheromone was present, the females did perform more head movements with a large-crested male than with a small-crested male (2, Table 7.9; Fig 7.7).

It seems that when pheromone was present no discrimination between large- and small-crested males was shown by the females in their performance of head movements, but discrimination did occur when no pheromone was present. It is not immediately obvious why this should be so, but since the role of this particular

behavioural action is as yet undetermined, this result can only be discussed speculatively (see Section 7.6).

In addition, the examination of order effects yielded some interesting results as shown in Table 7.10 and Fig 7.8.

1. Females perform more head mvmts with 2nd male present than with 1st (+ph) (Wlcn) $p < 0.05$
2. Females perform more head mvmts with 1st male present than with 2nd (-ph) (Wlcn) NS
3. Females perform more head mvmts with ph present than when it is absent (1st m) (t-test) $p < 0.05$
4. Females perform more head mvmts with ph present than when it is absent (2nd m) (t-test) $p < 0.01$

Table 7.10 Hypotheses tested to investigate order effects in no.s head movements performed in visual stimulus presentation period. Ph = pheromone; mvmts = movements; Wlcn = Wilcoxon test; NS = not significant.

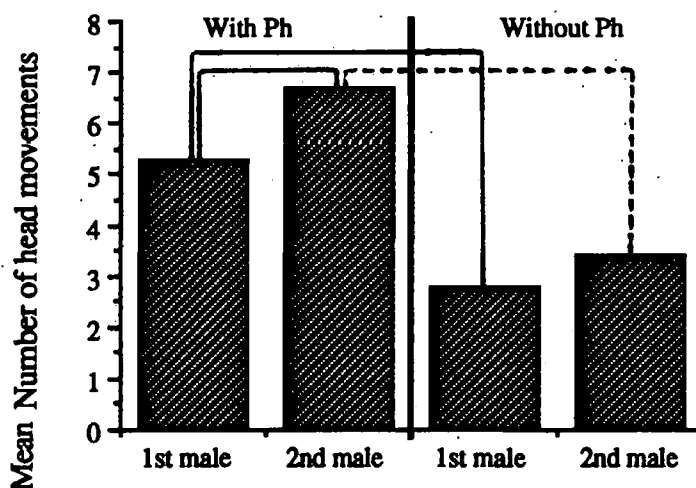


Fig 7.8 No. head movements performed by females in visual stimulus presentation period. Ph = pheromone; — = $p < 0.05$; = $p < 0.01$. (For with ph, $N = 17$; for without ph, $N = 16$.)

Results 3 and 4 in Table 7.10 indicate once again that the presence of pheromone meant that females performed more head movements than they did when no pheromone was present (see also Fig 7.8). Interestingly, in the presence of

pheromone, females performed more head movements when the second visual stimulus male was presented compared with when the first male was presented (1, Table 7.10; Fig 7.8). This indicates that again, as also shown in Section 7.5.8, females respond differently to the presentation of the first visual stimulus male compared with the presentation of the second; in this case the response is manifested by the females performing a greater number of head movements with the second male.

It is also interesting that in the absence of pheromone no difference in number of head movements was shown between the first and second males presented (2, Table 7.10; Fig 7.8). This, when considered in the light of result 2 (Table 7.9) (in the absence of pheromone females performed more head movements in the presence of a large-crested male than with a small-crested male), suggests that the large crest on the male may be the visual stimulus to which the females responded by performing more head movements. Thus the presence of pheromone, the presentation of the first of the visual stimulus males and the presentation of a large-crested male all made a difference to the number of head movements the females performed.

7.5.9 The visual stimulus presentation period - *number of open-mouth sniffs*

Very few open-mouth sniffs were recorded in the visual stimulus presentation period. As with the data for the olfactory stimulus presentation period the hypotheses (see Section 7.4.3) could not be tested so the actual numbers of females performing open-mouth sniffs are considered here (see Table 7.11).

With Pheromone	Without Pheromone
8/17 (47.1%) - 1st test	5/17 (29.4%) - 1st test
6/17 (35.3%) - 2nd test	4/17 (23.5%) - 2nd test

Table 7.11 No.s of females performing open-mouth sniffs in visual stimulus presentation period.

As for the olfactory stimulus presentation period there seem to be fewer open-mouth sniffs when pheromone is absent, the data are too few for statistical analysis however and no order effects were apparent.

7.5.10 The visual stimulus presentation period - *number of boundaries crossed*

The hypotheses outlined in Section 7.4.3 were tested, the results are shown below (Table 7.12).

1.g)Females cross fewer boundaries with a LCr m than with a SCr m (+ph)	(Wlcn) NS
2.g)Females cross fewer boundaries with a LCr m than with a SCr m (-ph)	(Wlcn) NS
3.h)Females cross fewer boundaries when ph is present than when absent (LCr)	(t-test) NS
4.h)Females cross fewer boundaries when ph is present than when absent (SCr)	(t-test) NS

Table 7.12 Hypotheses tested and results for no. of boundaries crossed in visual stimulus presentation period. Ph = pheromone; LCr = large-crested; SCr = small-crested; m = male; Wlcn = Wilcoxon test; NS = not significant.

The results indicate that neither presence nor absence of pheromone or the size of the visual stimulus male's crest made a difference to the females' level of activity (measured as the number of boundaries crossed). Similarly, when order effects were considered (see Table 7.13), neither presence nor absence of pheromone or whether it was the first or second male that was presented made a difference to the females' level of activity.

1.Females cross fewer boundaries with 1st male than with 2nd male (+ph)	(Wlcn) NS
2.Females cross fewer boundaries with 1st male than with 2nd male (-ph)	(Wlcn) NS
3.Females cross fewer boundaries when ph is present than when it is absent (1st male)	(t-test) NS
4.Females cross fewer boundaries when ph is present than when it is absent (2nd male)	(t-test) NS

Table 7.13 Hypotheses tested to investigate order effects in the no. of boundaries crossed in visual stimulus presentation period. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

Contrary to expectation, these experiments provide no evidence for the hypotheses that females show lower activity levels when pheromone is present or the visual stimulus of a male is present. Thus it seems that females do not demonstrate their interest in a male or in mating by moving around less than when they have no such interest.

7.5.11 The visual stimulus presentation period - *time spent near*

The hypotheses outlined in Section 7.4.3 are based on the idea that females might show discrimination between males by spending more time in close proximity to a 'preferred' male (Verrell 1983). Originally the data for each of the two categories 'near' and 'very near' were to be treated separately but the two were combined as 'near' to ensure that there were enough data for the appropriate analysis. (Because the females did not move around as much as expected they often did not spend any time in one or more of the particular categories. This meant that certain females would have had to be eliminated from the analysis, so the data were assigned to only two categories 'near' and 'far', instead of the original four.) The results are shown in Table 7.14.

1.i) Females spend more time 'near' to a Lcr male than a SCr male (+ph)	(Wlcn) NS
2.i) Females spend more time 'near' to a Lcr male than a ScCr male (-ph)	(Wlcn) NS
3.j) Females spend more time 'near' when ph is present than when absent (Lcr)	(t-test) NS
4.j) Females spend more time 'near' when ph is present than when absent (ScCr)	(t-test) NS

Table 7.14 Hypotheses tested and results for time spent near male in visual stimulus presentation period. Ph = pheromone; Lcr = large-crested; ScCr = small-crested; Wlcn = Wilcoxon test; NS = not significant.

From the results (Table 7.14) it appears that females did not spend more time with a male of a particular crest height and this was not affected by the presence or absence of pheromone. In addition, there were no effects with regard to the order of

presentation of the visual stimulus males, nor was this affected by the presence or absence of pheromone (see Table 7.15).

1.Females spend more time 'near' to the 1st male than the 2nd (+ph)	(Wlcn) NS
2.Females spend more time 'near' to the 1st male than the 2nd (-ph)	(Wlcn) NS
3.Females spend more time 'near' a male when ph is present than absent (1st male)	(t-test) NS
4.Females spend more time 'near' a male when ph is present than absent (2nd male)	(t-test) NS

Table 7.15 Hypotheses tested to investigate order effects in time spent near in visual stimulus presentation period. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

Thus females did not show any discrimination between males by their proximity to a particular male, at least in terms of the 'near' category used here (i.e. in the same half of the observation tank. The presentation of visual stimuli to the females did not appear to alter their behaviour in terms of spending more time in the same area as a particular male.

The final sections of the results go on to examine more closely the issue of proximity of the females to the males presented to them.

7.5.12 The visual stimulus presentation period - *time spent within 10cm*

In the hypotheses tested in this section (see Section 7.4.3), the importance of a distance of approximately one body-length between the female and the visual stimulus male was investigated. Table 7.16 lists the results.

1.k)Females spend more time within 10cm of a LCr than a SCr male (+ph)	(Wlcn) NS
2.k)Females spend more time within 10cm of a LCr than a SCr male (-ph)	(Wlcn) NS
3.l)Females spend more time within 10cm of male when ph is present than absent (LCr)	(t-test) NS
4.l)Females spend more time within 10cm of male when ph is present than absent (SCr)	(t-test) NS

Table 7.16 Hypotheses tested and results for time spent within 10cm of male in visual stimulus presentation period. Ph = pheromone; LCr = large-crested; SCr = small-crested; Wlcn = Wilcoxon test; NS = not significant.

Females did not spend more time within ten cm of either male, nor did the presence of pheromone make any difference to the females' behaviour. Also, when order effects were looked at (see Table 7.17), no significant results emerged here either.

1.Females spend more time within 10cm of 1st male than 2nd male (+ph)	(Wlcn) NS
2.Females spend more time within 10cm of 1st male than 2nd male (-ph)	(Wlcn) NS
3.Females spend more time within 10cm of male when ph is present than absent (1st m)	(t-test) NS
4.Females spend more time within 10cm of male when ph is present than absent (2nd m)	(t-test) NS

Table 7.17 Hypotheses tested to investigate order effects in time spent within 10cm of male in visual stimulus presentation period. Ph = pheromone; m = male; Wlcn = Wilcoxon test; NS = not significant.

Females did not appear to discriminate between males by spending more time within a body-length (ten cm) of a particular male, nor did the presence of pheromone seem to affect this.

In the final Section below, the rates of sniffing by the female when within ten cm and not within ten cm of the male were investigated.

7.5.13 The visual stimulus presentation period - *rates of sniffing*

The different hypotheses relating to whether females sniff faster with a large-crested male than with a small-crested one under different conditions are detailed below (Table 7.18; Figs 7.9 and 7.10). N.B. wt = within ten cm; nwt = not within ten cm.

- | | |
|---|-------------------|
| 1.m) Females sniff faster with LCr male than with SCr male (+ph) (wt) | (Wlcn) $p < 0.05$ |
| 2.m) Females sniff faster with LcCr male than with SCr male (+ph) (nwt) | (Wlcn) NS |
| 3.m) Females sniff faster with LCr male than with SCr male (-ph) (wt) | (Wlcn) NS |
| 4.m) Females sniff faster with LCr male than with SCr male (-ph) (nwt) | (Wlcn) NS |

Table 7.18 Hypotheses tested and results for comparing rates of sniffing between LCr and SCr males.

Ph = pheromone; LCr = large-crested; SCr = small-crested; wt = within ten cm; nwt = not within ten cm; Wlcn = Wilcoxon test; NS = not significant.

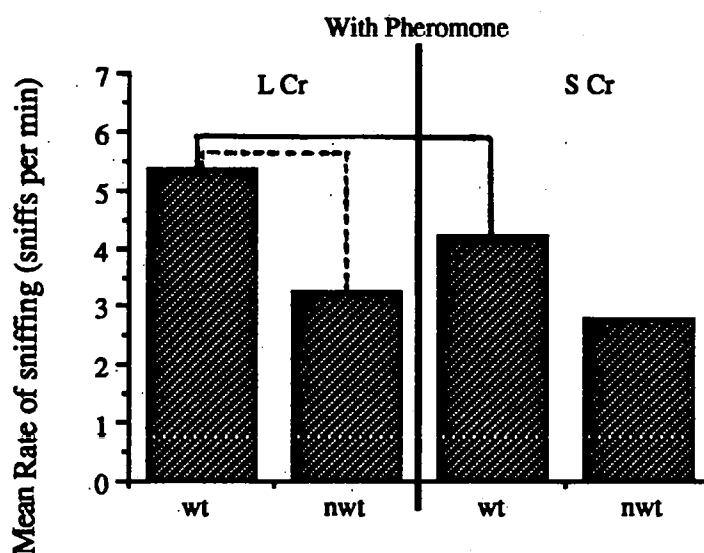


Fig 7.9 Rate of sniffing by females wt and nwt of males for LCr and SCr males. LCr = large-crested; SCr = small-crested; wt = within ten cm; nwt = not within ten cm. (For with pheromone, N=11) --- = $P < 0.05$, = $P < 0.01$.

When pheromone was present, females sniffed at a faster rate when presented with a large-crested male than with a small-crested male, but only when within ten cm of the male (1, Table 7.18; Fig 7.9), not when not within ten cm (2, Table 7.18; Fig 7.9). However, when pheromone was absent, this distinction between large- and small-crested males did not occur, neither when within ten cm (3, Table 7.18; Fig 7.10) nor when not within ten cm (4, Table 7.18; Fig 7.10).

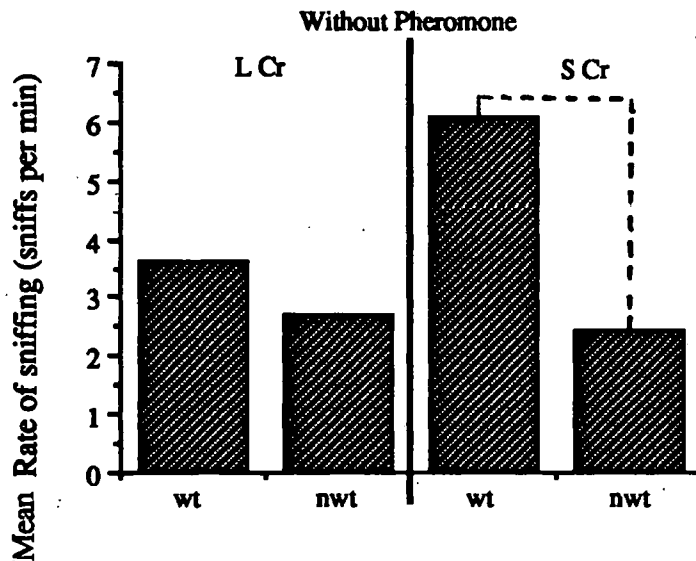


Fig 7.10 rate of sniffing by females wt and nwt of males for LCr and S Cr males LCr = large-crested; S Cr = small-crested; wt = within ten cm; nwt = not within ten cm. (For without pheromone, N=11;.....=P<0.01.)

These results suggest that the prior exposure to pheromone and the continued exposure to it during the presentation of the visual stimulus enabled the females to make a discrimination between the large- and small-crested male, by sniffing at a faster rate with the large-crested male. In addition, this discrimination was only evident when the female was within ten cm of the male.

The results for the hypotheses looking at whether females sniff faster when pheromone was present than when it was absent are shown in Table 7.19.

1.n)Females sniff faster when ph is present than when absent (Lcr) (wt)	(t-test) NS
2.n)Females sniff faster when ph is present than when absent (Lcr) (nwt)	(t-test) NS
3.n)Females sniff faster when ph is present than when absent (Scr) (wt)	(t-test) NS
4.n)Females sniff faster when ph is present than when absent (Scr) (nwt)	(t-test) NS

Table 7.19 Hypotheses tested and results for comparing rates of sniffing by females with pheromone present and absent. Ph = pheromone; Lcr = large-crested; Scr = small-crested; wt = within ten cm; nwt = not within ten cm; NS = not significant.

Under none of the conditions tested did the females sniff faster in the presence of pheromone than in its absence. This result might well be expected in the light of earlier results (see Section 7.5.2 and 7.5.5). These suggested that the females had become sensitised to the pheromone by the later stages of the experimental protocol and did not respond to pheromone in the water any differently than they did to plain water.

The different hypotheses investigating whether females sniff faster when within ten cm of the visual stimulus male in different conditions are shown in Table 7.20 and Figs 7.9 and 7.10.

1.o) Females sniff faster when wt of stimulus male than nwt of him (+ph) (Lcr)	(Wlcn) $p=0.01$
2.o) Females sniff faster when wt of stimulus male than nwt of him (+ph) (Scr)	(Wlcn) NS
3.o) Females sniff faster when wt of stimulus male than nwt of him (-ph) (Lcr)	(Wlcn) NS
4.o) Females sniff faster when wt of stimulus male than nwt of him (-ph) (Scr)	(Wlcn) $p=0.01$

Table 7.20 Hypotheses tested for comparing rates of sniffing by females when wt of stimulus males and nwt of stimulus males. Ph = pheromone; Lcr = large-crested; Scr = small-crested; wt = within ten cm; nwt = not within ten cm; Wlcn = Wilcoxon test; NS = not significant.

When pheromone was present and the large-crested male was presented the females sniffed at a faster rate when within ten cm than not within ten cm (1, Table 7.20; Fig 7.9). This discrimination did not occur when the small-crested male was presented (2, Table 7.20; Fig 7.9). However, when no pheromone was present, females did not sniff faster when within ten cm than not within ten cm of the large-crested male (3, Table 7.20; Fig 7.10), but with the small-crested male they did sniff faster within ten cm than not within ten cm (3, Table 7.20; Fig 7.10). Here females seemed to show some discrimination by sniffing faster when within ten cm of the small-crested male than when not within ten cm.

When order effects were investigated some interesting results again emerged.

Table 7.21 and Figs 7.11 and 7.12 show the results of comparing sniff rates between the first and second stimulus males, under different conditions.

- | | |
|---|-------------------|
| 1.Females sniff faster with the 1st male than with the 2nd male (+ph) (wt) | (Wlcn) NS |
| 2.Females sniff faster with the 1st male than with the 2nd male (+ph) (nwt) | (Wlcn) $p < 0.05$ |
| 3.Females sniff faster with the 1st male than with the 2nd male (-ph) (wt) | (Wlcn) NS |
| 4.Females sniff faster with the 1st male than with the 2nd male (-ph) (nwt) | (Wlcn) $p < 0.05$ |

Table 7.21 Hypotheses tested to examine order effects for sniff rates between 1st and 2nd males. Ph = pheromone; wt = within ten cm; nwt = not within ten cm; Wlcn = Wilcoxon test; NS = not significant.

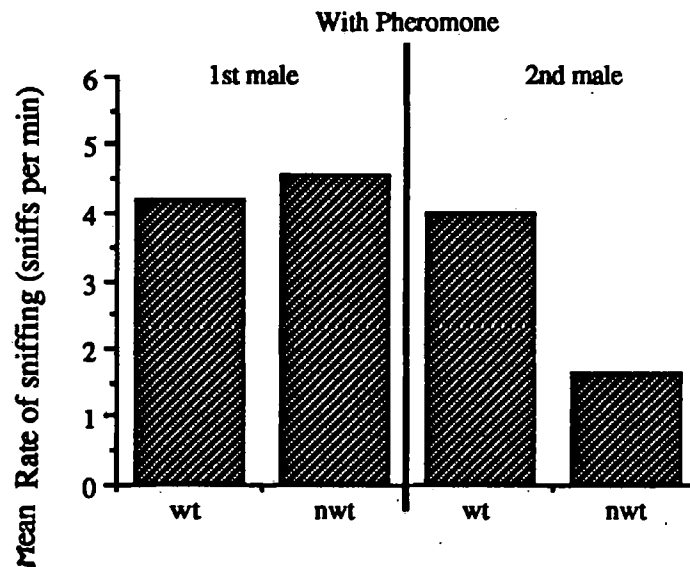


Fig 7.11 Rate of sniffing within ten cm and not within ten cm for 1st and 2nd males. Wt = within ten cm; nwt = not within ten cm. (For with pheromone $N=8$; ---= $p < 0.05$.)

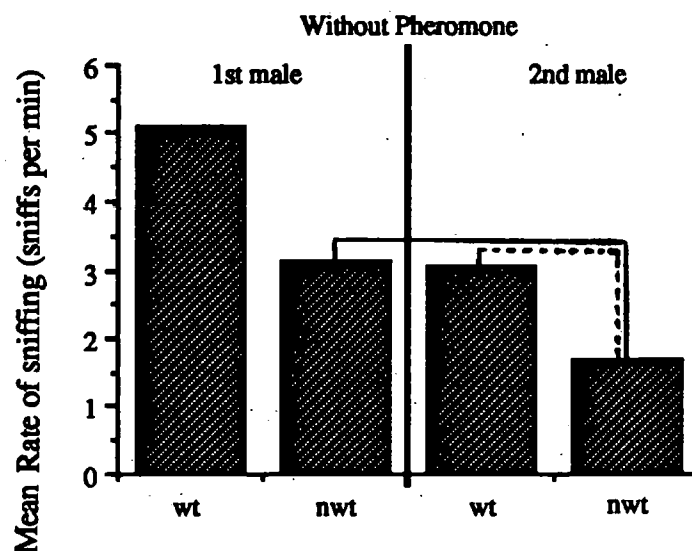


Fig 7.12 Rate of sniffing within ten cm and not within ten cm for 1st and 2nd males. Wt = within ten cm; nwt = not within ten cm. (For without pheromone N=8; ---=p<0.05;=p<0.01.)

When pheromone was present, females did not sniff faster with the first male than with the second when within ten cm of him (1, Table 7.21; Fig 7.11) but they did when not within ten cm (2, Table 7.21; Fig 7.11). Similarly, when pheromone was not present the same pattern emerged - when within ten cm they did not sniff faster with the first male (3, Table 7.21; Fig 7.12) but when not within ten they did sniff faster with the first male (4, Table 7.21; Fig 7.12).

When rates of sniffing in the presence and absence of pheromone were compared under different conditions no significant results were obtained (Table 7.22).

- | | |
|---|-------------|
| 1.Females sniff faster with pheromone present than when absent (1st male) (wt) | (t-test) NS |
| 2.Females sniff faster with pheromone present than when absent (1st male) (nwt) | (t-test) NS |
| 3.Females sniff faster with pheromone present than when absent (2nd male) (wt) | (t-test) NS |
| 4.Females sniff faster with pheromone present than when absent (2nd male) (nwt) | (t-test) NS |

Table 7.22 Hypotheses tested to examine order effects for sniffs rates in presence and absence of pheromone. Wt = within ten cm; nwt = not within ten cm; NS = not significant.

As explained earlier, this is probably because the females had become used to the pheromone.

The order effects for rates of sniffing when within ten cm and not within ten cm of a stimulus male under different conditions are shown in Table 7.23, Figs 7.11 and 7.12.

1.Females sniff faster when within ten cm of male than when not (+ph) (1st male)	(Wlcn) NS
2.Females sniff faster when within ten cm of male than when not (+ph) (2nd male)	(Wlcn) p<0.05
3.Females sniff faster when within ten cm of male than when not (-ph) (1st male)	(Wlcn) NS
4.Females sniff faster when within ten cm of male than when not (-ph) (2nd male)	(Wlcn) p<0.01

Table 7.23 Hypotheses tested to examine order effects for sniff rates within ten cm and not within ten cm of stimulus male. Ph = pheromone; Wlcn = Wilcoxon test; NS = not significant.

Both in the presence (2, Table 7.23; Fig 7.11) and absence (4, Table 7.23; Fig 7.12) of pheromone the females sniffed faster when within ten cm than not within ten cm of the second male, but this effect did not occur for the first male (1, 3, Table 7.23; Figs 7.11, 7.12). These results, together with 2 and 4 in Table 7.21 (see Figs 7.11, 7.12), suggest that the females rate of sniffing was high for the first male both when within ten cm and not within ten cm. By the time of the second test with the second male, the rate of sniffing was high only for when the female was actually within ten cm, but not when not within ten cm (2, 4, Table 7.23). This effect was the same when pheromone was present and when it was absent (see Table 7.23). Thus the females responded differently to the first male presented compared with the response to the second male and this was not affected by the presence of pheromone.

7.7 Discussion

7.7.1 Main conclusions from olfactory stimulus presentation period

The primary aim of this experiment was to determine the effect on the female and the importance to her of the olfactory and visual courtship cues provided by the

male. In the olfactory stimulus presentation period female behaviour in the presence and absence of pheromone was compared in detail to see if its presence alone (i.e. with no visual cues) had any effect. The conclusions are summarised below.

Looking at number of sniffs performed (Section 7.5.2) it was found that the presence of pheromone did make a definite difference to the females' behaviour; they performed more sniffs in its presence than in its absence. However, after about fifteen minutes had elapsed (the approximate time taken to get on to the second test), and the females had had some exposure to visual stimuli, they seemed to no longer respond to the pheromone in a marked way. (In addition, the females responded initially to a novel environment by sniffing at a high level, whether pheromone was present or not.)

Another difference that presence of pheromone made to the females' behaviour was the performance of a larger number of head movements. The number of open-mouth sniffs and numbers of boundaries crossed were not, however, found to be affected by the presence of pheromone. Finally, the orientating behaviour that the females performed (see Section 7.5.7) occurred more often in the presence of pheromone than in its absence.

Thus it seems that the female does respond to the putative courtship pheromone the male produces and that it has the capacity to cause changes in her behaviour.

7.7.2 Main conclusions from visual stimulus presentation period

This part of the experiment sought to investigate whether females would make any discrimination between the visual stimulus of a large-crested male and a small-crested male. In addition the effect of prior exposure to pheromone and exposure to it during the test was investigated to see if there is any interaction between the two sets of cues.

There is very little evidence that females make any discrimination between large- and small-crested males. One result showed that females performed more head

movements with a large-crested than with a small-crested male when no pheromone was present. Other results show that females make a distinction between males but only when pheromone is present also. For example, with the second males presented females perform more head movements than with the first, but only with pheromone present. Similarly, females sniff faster with a large-crested male than with a small-crested male, but only when within ten cm of him and with pheromone present. In these cases the pheromone alone does not have the same effect so it seems reasonable to conclude that the combination of cues is the important point in explaining these effects.

From previous work done (Verrell 1983) it was thought that it might be possible to detect discrimination of preferred males by using proximity as a measure of choice. However, the 'time spent near' and 'time spent within ten cm' parameters yielded no significant results. This brings out an important point about trying to detect mate choice. Recall that the females did make a discrimination by sniffing at a faster rate when within ten cm of a large-crested male even though no discrimination was detectable in terms of time spent within ten cm of him. It would seem important then to have a detailed knowledge of female behaviour to be able to detect discrimination that is not immediately obvious.

7.7.3 General Discussion

It is clear that olfaction plays an important role in the communication of newts and that vision may be less important than seems obvious to human perception. The results of this experiment suggest that olfactory cues are extremely important in the initial stages of courtship. The results also illustrate how complex the interaction between two individuals is in terms of the stimuli being presented by one sex and being received by the other. The interpretation of some of these results is difficult as it is not immediately obvious why a female should behave in the way observed in some of the results.

Two major areas are obvious candidates for further work. Firstly, the male's pheromone was found to affect the female's behaviour. More work needs to be directed at elucidating the composition of the pheromone (as has been attempted by Belvedere et al (1988) for other *Triturus* species). Then further work could be done to investigate more directly how the pheromone affects the female's behaviour. Secondly, the olfactory stimulus of a male newt was found to be more important to the female than a purely visual one; but the combination of cues was also important. Further work could now be directed at attempting to understand the exact mechanisms underlying the communication processes involved in newt courtship behaviour. This, as suggested by Ryan (1990), would considerably improve our understanding of mate choice and sexual selection.

CHAPTER 8

General Discussion

The work described in this thesis set out to examine the sexual behaviour of the female smooth newt. It has essentially taken the form of a study of female sexual responsiveness, approached from different perspectives and on different levels.

Longitudinal studies, observing individuals over an extended period of time, have been central to much of the work described here. They have revealed some important aspects of female behaviour (such as high sexual responsiveness early in the season) which had previously remained undetected. Also, by following individuals over time, the significance of certain behaviour patterns has been made clearer and put into a more natural context. Davies (1991) emphasises how important longitudinal studies can be for an understanding of the adaptive significance of behaviour. The costs and benefits of particular behaviour patterns may not be immediately apparent and only by following individuals through time might they be established.

Taking this longitudinal approach has also been of considerable value in trying to understand female sexual behaviour from two different perspectives, i.e. causal and functional. This is largely because the behaviour of the same individual females was examined simultaneously using the two approaches. Looking at the same behaviour from two perspectives at once can aid the understanding of each and have a synergistic influence on the understanding of the behaviour as a whole. Thus, a combination of functional and causal approaches can be used very effectively in a longitudinal study.

One approach underpinning all the work described here was to take the female's viewpoint in trying to understand both female behaviour patterns and newt sexual behaviour generally. It may seem pedantic to repeatedly emphasize this point but it is of fundamental importance to a true understanding of all aspects of animals' sexual behaviour. For example, careful consideration of the important factors in a female's reproductive strategy can make sense of behaviour patterns that do not, on

first appearance, seem particularly adaptive (e.g. see Chapter 4). Also, the recent studies that show variation in parameters measured in mate choice experiments (e.g. Zuk et al 1990) may be reflecting undetected variation in female responsiveness. Sullivan (1990) discusses this problem in terms of the females' sampling period.

It is sometimes difficult to recognise that a male oriented bias may exist in a particular field of study. However, once it has been identified, it is usually easy to see how even a perfunctory consideration of the female perspective can help throw light on the significance and importance of particular behaviour patterns. The discussion between Eberhard (1990) and Birkhead & Hunter (1990a; 1990b) on mechanisms of sperm competition illustrates these points well. A subtle shift in perspective can make a difference to our understanding of life-history strategies for each sex and this can alter our thoughts on the behaviour patterns that underlie the different mating systems.

The more experimental parts of the work discussed here (Chapter 7) also took a female perspective and, in addition, reflected the recent emphasis (e.g. Ryan 1990) on trying to understand how animals actually perceive each other. This has strengthened the attempt made in this work to try and understand the causal basis of female sexual motivation and choice behaviour. Once variations in female responsiveness had been detected and demonstrated (without any real consideration of the male's display input), then the potentially important aspects of male display could be identified and were investigated further.

In Chapter 1, the concept of motivation was discussed in some detail, particularly its usefulness to ethology. It was concluded that, provided the behaviour under study was carefully defined, the concept of motivation could have useful applications. Female sexual responsiveness was the behaviour investigated in this study and is, essentially, the behavioural manifestation of an individual's sexual motivation. Here, the use of the term 'motivation' has served to reinforce the fact that sexual behaviour is only one activity an animal may perform at any particular time, and that the animal is adapted to organise its activities. The phrase 'sexual motivation' also provides a useful shorthand way of referring to all those factors that contribute to the

expression, or not, of sexual responsiveness. Using the term motivation provides a common 'currency' for describing all an animal's activities, and allows them to be discussed in a broader context.

The major findings of the study will now be discussed in relation to each other, and with reference to Fig. 8.1, which summarises the main results of this work. Females were found to have variable sexual responsiveness under different conditions. In the study, the breeding season was divided into two parts, 'early' and 'later' (see Chapter 4). The threshold that the female's sexual motivation reaches before she progresses to the spermatophore transfer phase is shown in Fig 8.1. In this work female sexual responsiveness was described as being made up of two components: pre-display responsiveness and display responsiveness (see Chapter 5). Pre-display responsiveness is represented in Fig 8.1 by the solid curve. As shown in the figure, it was found to be high early in the season and fell to a lower level later on. Display responsiveness is represented by the dotted curves rising from the pre-display response curve. These represent the contribution to the female's responsiveness made by male display. This was found to be less important early in the season but became more so later on.

In the figure, two possible outcomes of a courtship encounter are shown, for both the early and later parts of the courtship. A single asterisk represents a courtship where spermatophore deposition occurred but no sperm was picked up. A double asterisk indicates that insemination took place. Early in the season, males in encounters where sperm was picked up did not have significantly higher tails than those where spermatophores were deposited, but no sperm was picked up. However, later in the season, they did have higher tails in encounters where insemination occurred.

Not shown in Fig 8.1 (for the sake of clarity) is the possible outcome of an encounter where the male does not achieve deposition. The results of this study showed that, both early and later in the season, females did show discrimination for males with larger crests at the deposition stage of courtship. That is, males that

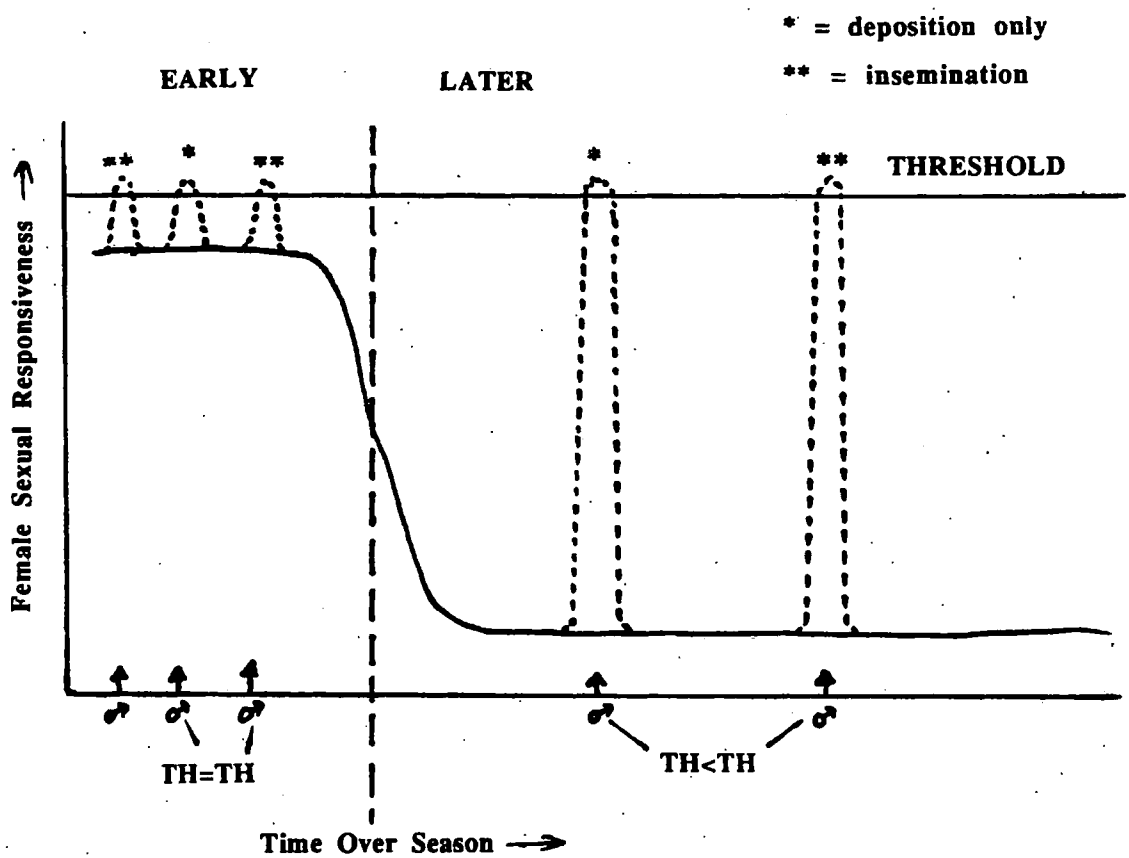


Fig. 8.1 Model of female sexual responsiveness. The threshold indicates the level of responsiveness when the female enters retreat display follow. The arrows and male symbols indicate incidences of courtship display. The solid curve represents the female's pre-display responsiveness; the dotted curves represent her display responsiveness. TH =tail height. (See text for details.)

achieved deposition (whether sperm was picked up or not) had significantly higher tails than those males that did not achieve deposition. Thus, females in this study mated multiply but they still showed some discrimination in their choice of males, especially later in the season.ig 8.1

This last point is interesting in the light of the results of Chapter 5. Later in the season, the male's display was found to be of greater importance (than it was early in the season) in raising the female's sexual responsiveness to reach the threshold for potential insemination. It is known that the rate at which males display affects female receptivity (Teyssedre & Halliday 1986). The results of Chapter 7 suggest that the visual cue of a static large-crested male, is not, in isolation, enough to elicit discrimination from the female. When this stimulus is present in combination with olfactory, tactile and the visual cues of movement, (coherently coordinated, as they are in courtship display) the female does, however, discriminate for large-crested males.

Considered together, these results suggest that the extent of development of a male's most obvious secondary sexual characteristic is related to his display rate. Thus females may be 'choosing' large-crested males by being selectively responsive to a high rate of courtship display. Whether this possible mechanism for choice could be described as active or passive is difficult to determine. Indeed, distinguishing a definition of active or passive choice is very difficult, and attempting to draw a distinction seems more to generate confusion than assist an understanding of mate choice (Arak 1983; Sullivan 1989).

This study emphasised the importance of considering the constraints on female sexual responsiveness. Egg-laying was found to be a major investment in terms of time, and probably is also in terms of energy. Egg-laying is itself limited by temperature, which also reduces activity generally. Recently, the wider constraints on the activities of animals have received much consideration (e.g. Endler 1987; Metcalfe et al 1987; Magnhagen 1990). This kind of work, coupled with the growing interest in investigating how animals really perceive each other, augers well for a welcome return to combined causal and functional approaches to the study of animal behaviour.

Areas for specific further study have been suggested at the appropriate stages in the thesis. Generally, an important first step would be to investigate the dynamics of the movements of individuals of both sexes, especially in a more natural situation than that found in an observation tank.

A second area that could now be fruitfully examined is the physiological mechanisms that underlie female responsiveness. Female behaviour patterns have now been defined and could be used to investigate female sexual responsiveness under different conditions. Related to this, more work could now be directed at the possible mechanisms involved in multiple mating and its relationship with responsiveness.

Finally, the interface between sexual motivation and mate choice studies has much potential. For example, the study by Teyssedre & Halliday (1986) could be revisited and the effect of male display on *specific* female behaviour patterns examined more closely. With further work on how females perceive the stimuli presented by the male in courtship, a much fuller understanding of female mate choice in this species could be obtained.

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